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Proceedings of the 1986 Sugar Processing Research Conference October 19-21, 1986 Savannah, Georgia

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PREFACE

This 1986 Sugar Processing Research Conference is one of a series of conferences held in alternate years to provide a forum for exchange of information among technical leaders of the sugar industry and to report on new and noteworthy developments.

The program for this conference was arranged by Margaret A. Clarke and Mary An Godshall. The conference coordinator was Shirley T. Saucier. These proceedings were edited by Mary An Godshall. The editorial assistant was Jacqueline E. Smith.

The series Proceedings of the Sugar Processing Research Conference, of which this is the third issue, continues the Proceedings of the Technical Sessions on Cane Sugar Refining Research, which was published every other year from 1964 to 1980. For individual copies of this volume as well as back issues of the former series as long as the supply lasts, write to Sugar Processing Research, Inc., P. O. Box 19687, New Orleans, Louisiana 70179.

Copies of this issue are also for sale at the National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161.

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Margaret A. Clarke
Managing Director
Sugar Processing Research, Inc.

Neil L. Pennington
President
Sugar Processing Research, Inc.

Frederick W. Parrish
Designated Representative
U.S. Department of Agriculture

CONTENTS

Events in sugar crystallization: S.P.R.I. Science Award Presentation Andrew VanHook	1
Sucrose crystal deformation caused by impurities in refinery and raw house products J. Bruijn and P.G. Morel du Boil	12
Crystallization studies in low grade syrup Raymond E. Dickey, Joseph F. Dowling, and Robert M. Morton	34
Recent laboratory studies at C & H Sugar Richard Riffer	54
Recent studies on dextrans and polysaccharides in refinery processes Margaret A. Clarke, Earl J. Roberts, and Thanh B.T. To	74
Mechanism of the alkaline degradation of monosaccharides J.M. de Bruijn, A.P.G. Kieboom, and H. van Bekkum	93
The production of liquid invert sugar with ion exchange resins R.W. Percival and J.E. Schuler	111
Some laboratory observations on the inversion of sucrose with strongly acidic cation exchange resins Mark Wnukowski and C. Chi Chou	119
The production of invert syrups using immobilized enzyme Michael J. Daniels	134
Product application research - a tool to support marketing Juha Ahvenainen and Juhani O. Kuusisto	149
Production and characterization of dextran from sugarcane juice Arthur W. Miller, Frederick W. Parrish, and Margaret A. Clarke	159
Studies of the colour of U.K. beet white sugar N.W. Broughton, D. Sargent, B.J. Houghton, and A. Sissons	174
Flavors from beet and cane sugar products Mary An Godshall	210
SURE: A new sugar decolorization process Dieter Frank, Lincoln D. Metcalfe, and John Park	230
Evaluation of carbon-char admixtures Brian Dewar and Andrew Ho	255
Integrating laboratory instruments in the process control lab J.C. Thompson and W.J. Frazee	280

Recent observations on sugar colorants in cane sugar
refineries

Margaret A. Clarke and Rebeca S. Blanco 292

Non-starch, soluble polysaccharides of sugarcane

Margaret A. Clarke, Earl J. Roberts, and Mary An Godshall 309

Gel-like material produced in the processing of coloured liquors

Richard A. Kitchen, Margaret A. Clarke, and A.J. DeLucca II 321

The use of reversed-phase chromatography in carbohydrate analysis

Eero Rajakyla 347

HPLC analysis of carbohydrates: Comparison of detectors and
evaluation of recent developments

W.S. Charles Tsang, Margaret A. Clarke, and Marta M. Valdes 372

EVENTS IN SUGAR CRYSTALLIZATION: S.P.R.I. SCIENCE AWARD PRESENTATION

Andrew VanHook

Chemistry Department, Holy Cross College, Worcester, Massachusetts

I am most appreciative of this honor you bestow upon me for something I have enjoyed doing for almost 50 years. As you know, I have never had any direct affiliation with any sugar house refinery or sugar research institute. My professional connection, after a period of unrelated industries, has been exclusively the teaching of chemical engineering and physical chemistry in several schools around the country and for the past 40 years at Holy Cross College. This is a small New England liberal arts college run by the Jesuits and my tenure there has been most enjoyable.

Early in my career I became interested in sugar and I should tell you about this since it is probably the reason you recognized in the selection of this award. After this story of what I have done I should like to tell you what I am doing now and then what I am already thinking about doing next. To me, this last is one of the most important aspects of research—raising more questions and posing more problems than there are answers. I think it is important for the continuing vitality of any enterprise to pursue those unknowns and especially right now for our own sugar industry.

In the late 30's and early 40's I was teaching at Lafayette College, and Dr. Bingham there was trying to get me involved in problems of viscosity and fluidity. This was the time when Henry Eyring was applying his activation complex ideas to such physical processes, and on several visits to him at Princeton I became acquainted with a Dr. N. Thon who was planning to revise the International Critical Tables. This has not yet come to pass, and the original edition of 1933 still stands as a warehouse of physical and chemical information. Since I had already shown an interest in crystals, Dr. Thon asked me to collate data on the speeds of crystallization, and in so doing I came for the first time upon the data of Kucharenko on the "Velocity of Growth of Sugar Crystals From Pure and Impure Solutions." These data are remarkable for their thoroughness and completeness, and I was amazed that Kucharenko made no particular effort to explain his results beyond speculating somewhat upon the nucleation process. So, as an ambitious young physical chemist, imbued with the ideas of Gibbs, G.N. Lewis, Eyring, and others, I proceeded to theorize with Kucharenko's data.

The data are given as extensive tables and plots in which the supersaturation concentration is expressed in terms of weight percentages. I merely recalculated these as mole fractions, molality, and molarity, of which the last is probably most suitable whenever transport is involved. However, density data at the high temperature and concentrations of sugar boiling are meager, so that the other scales are used more commonly. Since the three modes are almost directly proportional one to another (1), it makes little difference for purposes of interpreting the mechanism of reaction.

On the molal scale, then, the outstanding feature of Kucharenko's curves is that growth rate increases only slightly more than linearly as supersaturation becomes greater, more markedly at lower temperatures. Also, in dilute solutions, so does the activity, chemical potential, effective concentration, or driving force to give it some of its many names. So, I put the two together and proposed the activity as the driving force of crystallization rather than the usual molecular concentration. Unfortunately, activity information in the region of saturation and beyond was not available, so I extrapolated from the more dilute concentrations--a procedure I deny to my students but yet use myself--and drew up a reasonable unifying account of Kucharenko's data. This thermodynamic rationalization has since been elaborated for sugar solutions by Professor Dunning of Bristol, by Naveau and Heitz, and most recently by Aquilamo in Italy, as well as by Mullin in London for other crystallizing systems. Saska, of the Audubon Sugar Institute, has also used this concept in some of his work.

A bothersome feature during this analysis was the fact that the agreement at higher temperature, above 50°C (typical of factory work) was not as good as at lower, more convenient laboratory conditions. This led us to investigate the temperature coefficient of crystallization, and our results as well as those of others can be summarized by the activation energy versus temperature plot of figure 1.

The inconstancy means that there is more than one step involved in building a crystal. At the higher temperature it is the transport of molecules from the bulk of the solution to the growing interface which is the slower and therefore rate controlling part, while at the lower temperature the incorporation of these molecules into the lattice itself is dominating.

On this score, Professor Dedek (4) calculated the rate at which molecules would arrive by diffusion at a planar surface and found that at 50°C or above it is just about the same as growth rate but increasingly faster below. At that time, in the 1950's, when I was perhaps overemphasizing the relative unimportance of diffusion on this account, Mr. Ness of the Great Western Sugar Company was very encouraging to the idea which was somewhat repugnant to some sugar boilers. In this same regard I must pay my respects at this time

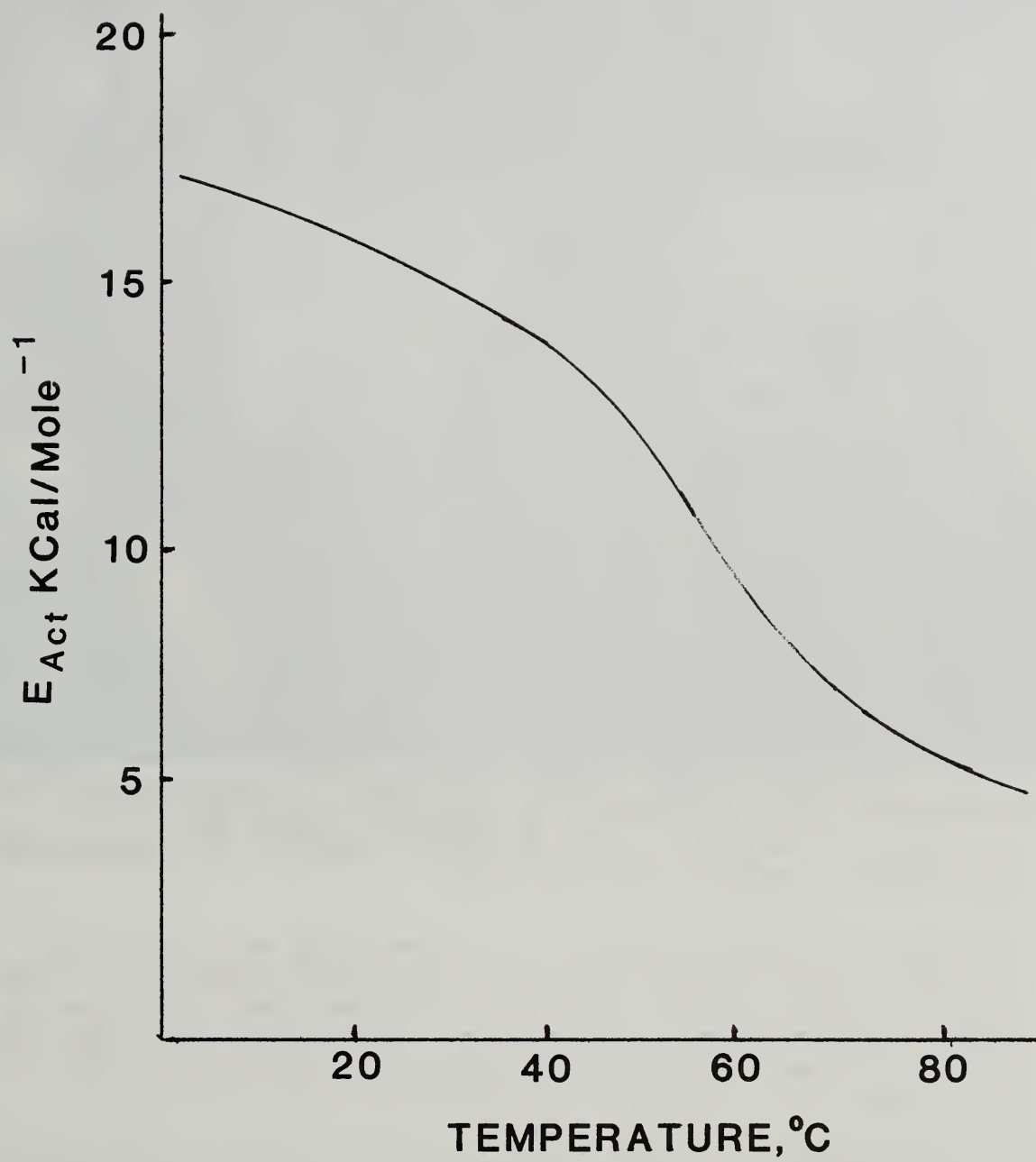


Figure 1.—Activation energy as a function of temperature.

to my mentors in sugar technology: Dr. Zerban, Hermann Hirschmuller, Dr. Honig, Harold Powers, Dan Gutleben (who honored me by calling me a "sugar tramp"), Al Webre, Ben Oxnard, and many, many others.

After establishing the role of diffusion, we went on to confirm the surface reaction part on the basis of the Burton-Cabrera-Frank theory (BCF). Mantovani (5) and his group at Ferrara have gone into this in great detail.

To move on from past to current work:

1. Surfactants:

I recently wrote a paper on surfactants in sugar crystallization. Our principal conclusions were first, that brighter and cleaner crystals appear favored when surfactant is present in the syrup, as demonstrated in figure 2.

This applies whether large monocrystals or small sizes are being grown in a batch—and especially so when conditions favor inclusions, a point I shall comment on presently.

As you know, the use of surfactants in sugar boiling has been demonstrated in low purity strikes. Surfactants have no real effect on growth rate; this is best ascertained with single crystals. But surfactants do promote secondary grain while yet preventing conglomeration. This can be observed microscopically as strike proceeds or in the growth curves illustrated in figure 3. These are only laboratory observations, but if duplicated in factory operation could be both disadvantageous and advantageous, especially for a continuous crystallization operation. The reduced boiling time for surfactants suggested by the graph is likely the net effect of secondary grain formation, absence of conglomerates, and enhanced lubricity in massecuite.

To look into this last factor in a superficial way, Brookfield viscosities of saturated syrup and a simulated massecuite of 50% crystal content, without and with surfactant, were determined at 60°C. No significant difference was noted in the first case, but a 10-20% increase in fluidity was obtained in the second. Why? I don't know.

2. Inclusions:

The next matter with which I have been recently concerned is inclusions. My general observations—nothing really new—are that to reduce them:

a) Surfactants are very helpful.



Figure 2.--Sucrose crystals grown from syrups without (left) surfactant and with (right) 300 ppm on water surfactant H.

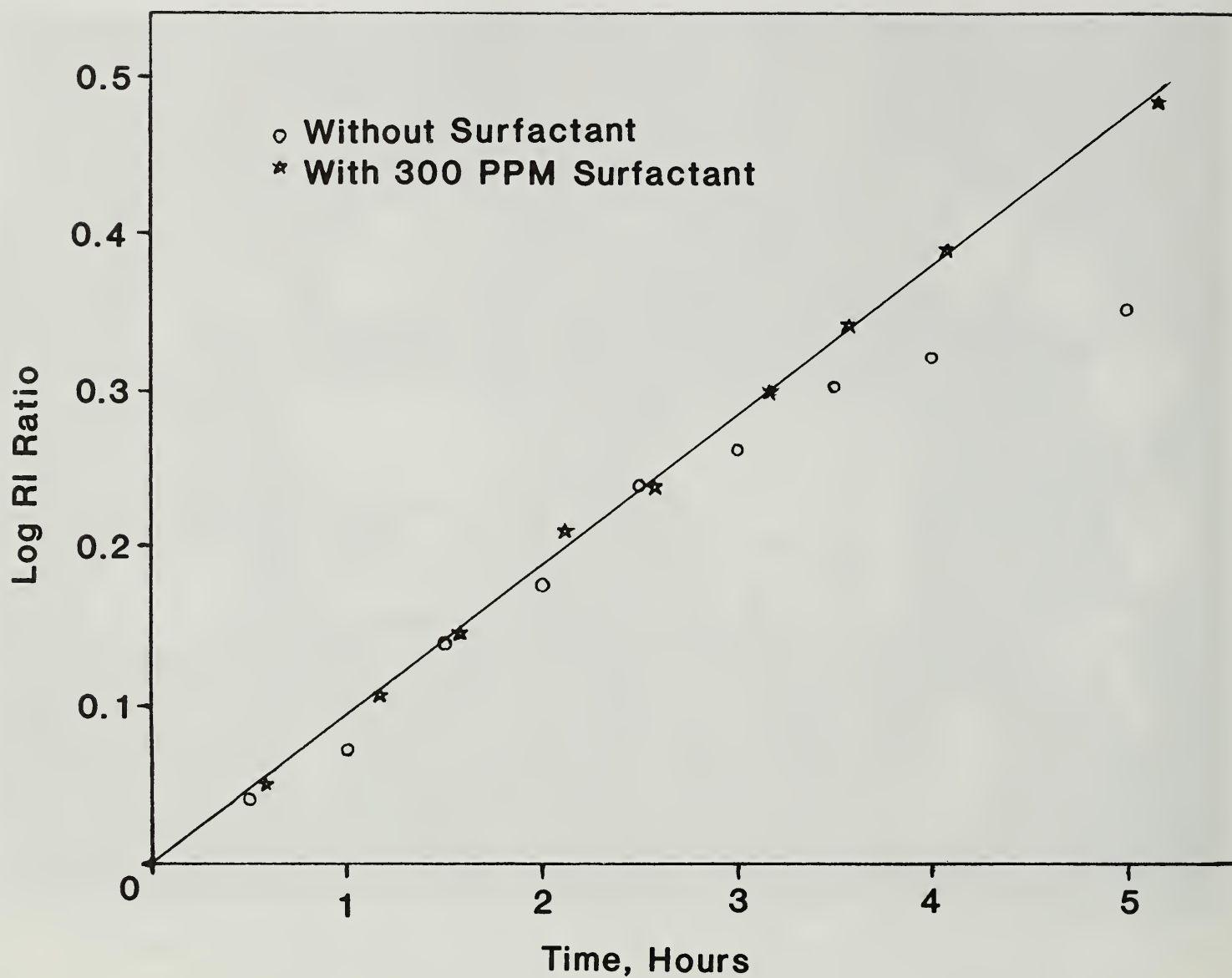


Figure 3.--Adjustment of seeded 1.1 syrup at 30°C.

b) Slow steady, and uninterrupted growth is absolutely necessary.

Best of all, follow a slow cooling schedule in order to avoid possible positive temperature excursions. These may lead to incipient etching and trapping of mother liquor upon subsequent growth.

c) Appropriate stirring would seem necessary to insure uniform growth conditions, yet the crystals illustrated in figure 4 were grown under static conditions. I wonder if growth in space would be helpful.

Ordinary inclusions move about within and even out of crystals under a mild temperature gradient such as imposed by spreading on a hot plate.

Details of these behaviors will be revealed in a paper soon to be published.

3. Triangles:

Habit modifications have been an ongoing subject for many years in many laboratories, including my own. Some time ago Bruce Rutherford of Fairymead in Queensland called my attention to the occurrence of triangularly shaped crystals, as shown in figure 5, A, in some low purity products. Of course, he whetted my curiosity.

With the limited sample on hand and having no pilot pan, I had to devise a simple screening test, which consisted essentially of diluting to about 25 Brix, centrifuging, and allowing to crystallize slowly at 60°C on a microscope slide or, better, in a covered petri dish. This does not reproduce the original but throws down a galaxy of small normal, elongated rodlike D crystals and distorted triangles. The simultaneous occurrence of the last two (see fig. 5, B) is very suggestive of a correlation between them.

In this way we ascertained:

1) Triangles, affined with excess saturated syrup, recrystallize in the same form, but the remaining syrup throws down mostly shapes and rocks. This, as well as other behaviors, suggests that the triangles have carried down by adsorption or inclusion the causative agent, and that their formation depends on the impurity/water and impurity/sugar ratio.

2) Ash itself is not the culprit and neither does lead clarification make much difference. Other common impurities were tried but none were effective. However, I feel that fructose may be implicated, and the possibility also remains that a combination of impurities may be required.

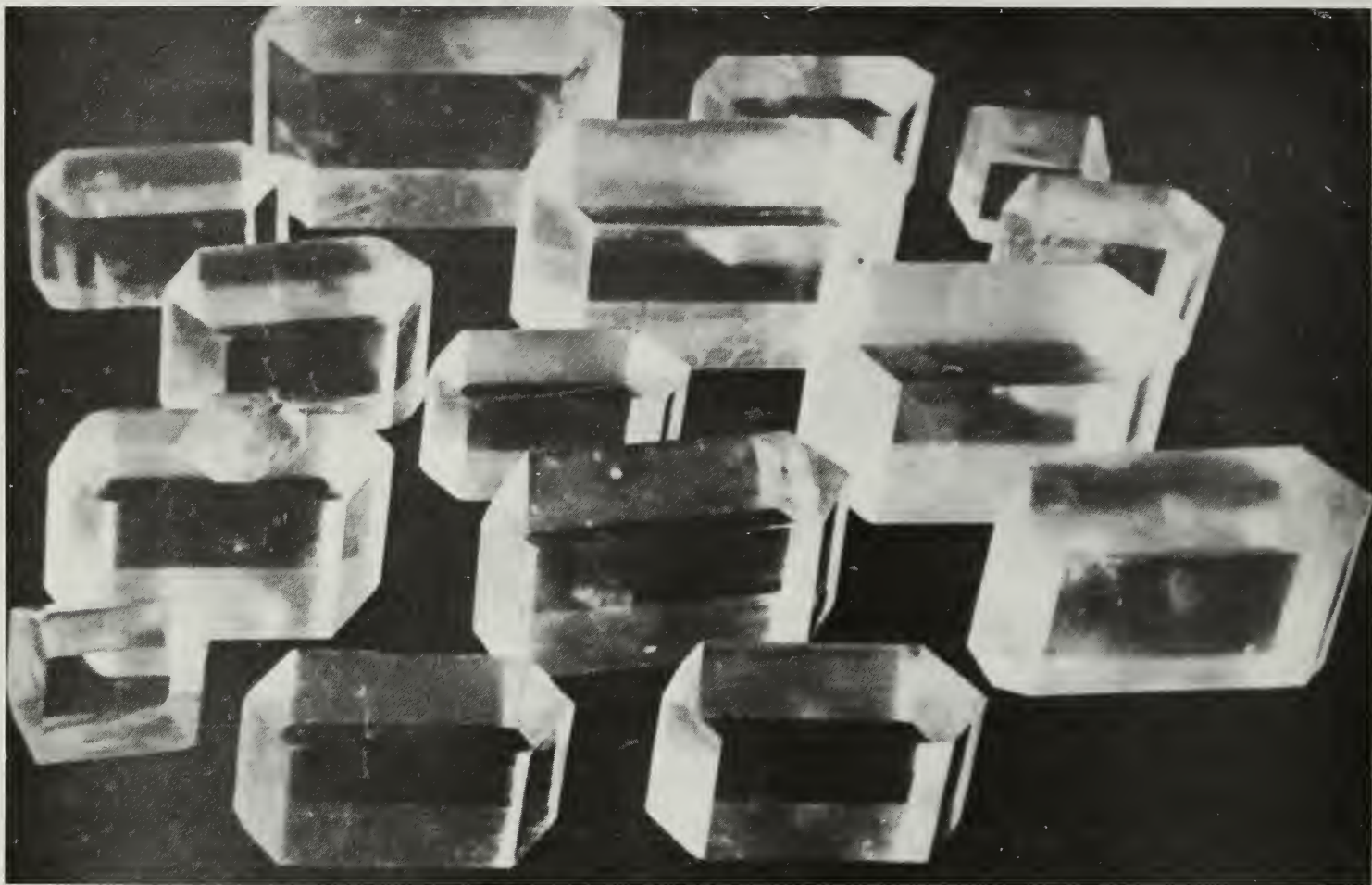


Figure 4.--Sucrose monocrystals.

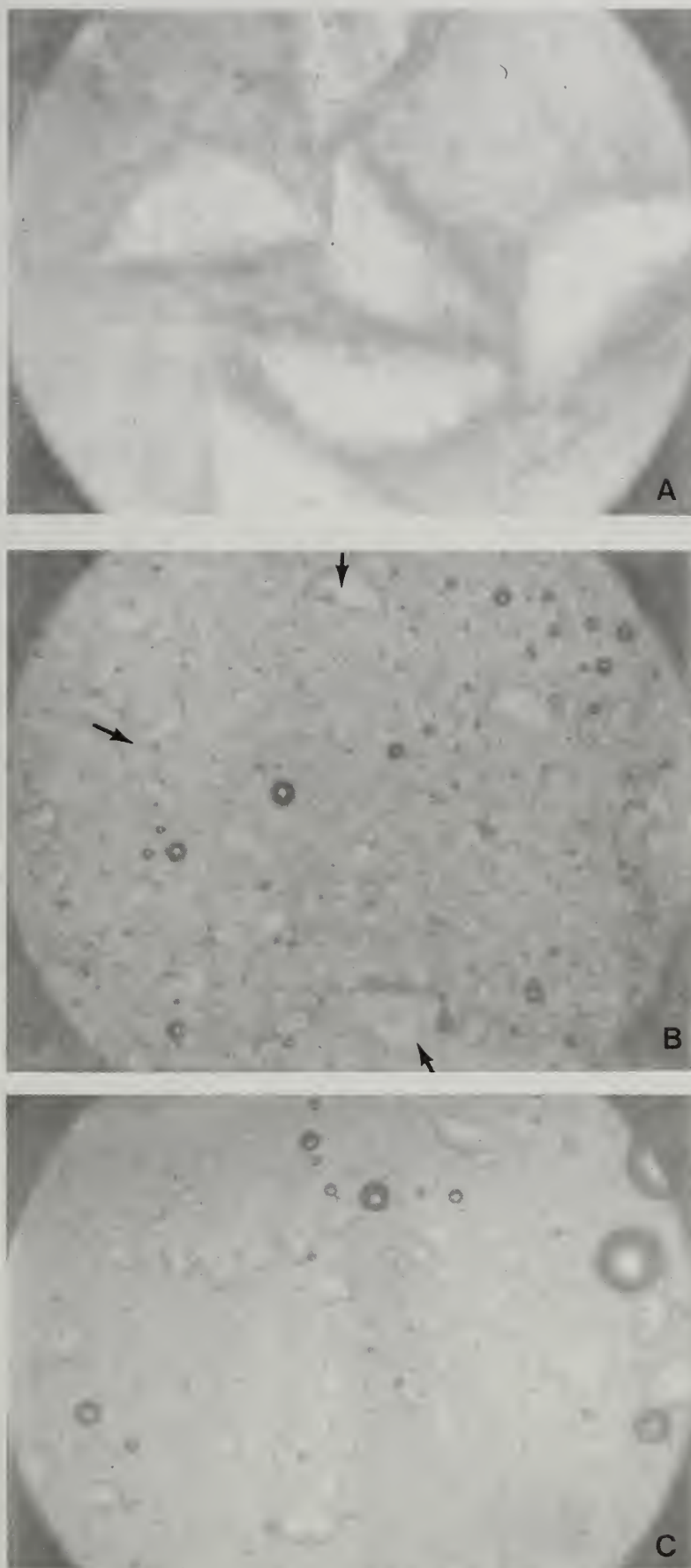


Figure 5.--Triangles: (A) Original massecuite, 60 X. (B) Positive screening test; molecular weight fraction <3500, 250 X. (C) Negative screening test; molecular weight fraction >13,000, 250 X.

- 3) Digestion at high (9-10) and low (4-5) pH does not destroy the triangle formation ability of the mixture.
- 4) Repeated wetting and recrystallization or temperature cycling seems to degrade triangles to rods and normal shaped crystals.
- 5) Both alcohol and dialysis fractionation indicate that it is the low molecular weight components (< 3500) and not the high which promote triangles; that is, oligomers rather than polysaccharides (see figs. 5, B, and 5, C).
- 6) Several enzymes were tried in a haphazard way as a preventive measure, but only one was attractive. This was SP 249 from Novo at 300 ppm at 25 Brix, pH 4-5, 60°C for 40 hours. The others tried were AMC and Dextranase from Novo, yeast diastase, and amylase.

Now, what am I doing next?

- a) There are problems to be solved; e.g., does a "good" crystal (3) dissolve slower than a "poor" one?
- b) Nucleation: Is the critical nucleus a perfect one? Does its shape influence the final habit?
- c) Hexoses: Are they involved in the hemimorphic properties of sucrose?

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DISCUSSION

Richard Riffer, C and H Sugar - Why is it that some impurities, such as raffinose, are much more rate-impeding than others?

VanHook - I think that those impurities poison the centers of growth.

Albert Villamil, Sugar Cane Growers Cooperative of Florida - What is the present status of the activity theory?

VanHook - It's not much different from what was 30 or 40 years ago. There's been some work by Bill Gunning (Tate and Lyle, Internal Report). I recently sent a paper to Zuckerindustrie pointing out the lack of data on the activity of sugar in solution. Acquisition of such data would be helpful not only at the laboratory level on studies of rate of growth, but also at the factory level. The data can be best acquired in the factory, observing not only the rate of growth of the crystal but also the boiling point rise. This is actually the basis on which we calculate the activity of the sugar.

Margaret A. Clarke, S.P.R.I. - With regard to your comments on crystallization in space: a few years ago, scientists at the Sugar Research Institute in Cuba (ICINAZ) were performing experiments on crystallization of sugar in space, using space vehicles, but I have not heard about their results.

VanHook - Such experiments have been performed with other crystals--fast growing crystals like alum, or disodium hydrogen phosphate, which grow much faster than sugar. Sucrose is a relatively slow growing crystal, about 10 times slower than most.

In space, without gravity, natural convection is diminished. In experiments on earth, it's inevitable that there is convection: if the crystal grows, there is generation of heat and that, no matter how small it is, creates convection currents. If these are minimized, growing conditions will be more uniform.

SUCROSE CRYSTAL DEFORMATION CAUSED BY IMPURITIES IN REFINERY AND RAW HOUSE PRODUCTS

J. Bruijn and P.G. Morel du Boil

Sugar Milling Research Institute

INTRODUCTION

The angles between crystal faces are characteristic and constant for a chemical compound. The outside appearance of crystals is influenced by conditions during growth, and the growth rate of the various crystal faces can vary. Growth rate is governed by supersaturation, temperature, circulation, viscosity and the concentration of impurities. The last factor exerts the main influence on crystal shape since certain impurities are preferentially adsorbed on some faces, causing these faces to grow more slowly than normal.

A sucrose crystal grown in pure aqueous solution is not square. The ratio between the length in the b-axis direction (y) and the length in the c-axis direction (z) is 2. This can be calculated from published data (Vernon 1938, Smythe 1967a) and was mentioned by Vane (1981). Crystals showing elongation in the c-direction have a value below 2 for this ratio while a higher value indicates elongation in the b-direction. Many substances have been reported to cause sucrose crystal habit modification. They can be separated into 3 groups

- polysaccharides
- oligosaccharides
- inorganic salts.

Of the first group, dextran, in particular, has been found to influence sucrose crystal shape. The Australians have published extensively in this area since the introduction of mechanical harvesting, which led to increased dextran concentrations in factory products.

Keniry (1967) found dextran a quantitative indicator

for the processing quality of cane and showed correlations between its concentration and crystal elongation. Sutherland (1968), Sutherland and Paton (1969) as well as Leonard and Richards (1969) separated syrups into oligosaccharide and polysaccharide fractions and concluded that the predominant impurity causing crystal elongation was a polysaccharide and that oligosaccharides had little influence. Later Day (1971) and Covacevitch (1977) demonstrated the effect of temperature, molecular mass of dextran, concentration and structure. The growing medium of these workers, however, was not a pure sucrose solution to which dextran had been added, but was A-molasses, which already contained various oligosaccharides.

Many authors have reported only slight elongation in the c-direction after adding dextran to pure sucrose solutions. Mantovani (1973) reported y/z ratios of 1.0 - 1.5 for sucrose crystals grown in supersaturated sucrose solution containing 5% dextran. Hidi and Staker (1975) decreased the dextran content in juice during factory trials from 1,700 ppm to 100 ppm using enzymic hydrolysis, but found that the y/z ratio of crystals only increased from 0.75 to 0.90. In contrast to this Inkerman (1980) found a marked reduction in elongated crystals after complete enzymic removal of dextran.

Saska and Polack (1982) investigated the effect of dextrans and partially hydrolysed dextrans on sucrose crystal habit and found that the elongating effect decreased as the molecular mass decreased. Cremata (1983) on the other hand stated that low molecular mass polysaccharides which formed between cutting and processing of sugar cane caused elongation in the c-axis direction.

In the West Indies Tilbury (1971) did not find any correlation between dextran content and crystal elongation in C-massequite during factory experiments. It must however be realised that dextran analyses are generally non-specific. Mantovani (1975) and Shah and Delavier (1974) observed cubic crystals in the presence of dextran which indicates that growth has been retarded in both the b- and c-directions.

The influence of oligosaccharides on sucrose crystal habit has been better documented. A classical example of b-axis elongation is that caused by raffinose. More recently Mantovani (1973) studied the effect of raffinose on crystal shape between 15°C and 45°C and found an increasing influence at higher temperatures.

Kamoda (1968) separated a polysaccharide and an oligosaccharide fraction from refinery molasses and found the latter the causative factor of sucrose crystal habit modification in laboratory experiments. Montenegro (1983) also found that oligosaccharides in factory products caused elongation in the c-direction.

Smythe (1967a, b, c) carried out extensive studies on crystallisation rate and habit modification in impure sucrose solutions. His third paper lists the influence of various oligosaccharides on elongation in the b- and c-directions.

Nurok (Anon 1971) carried out crystallisation experiments at the Sugar Milling Research Institute and concluded that the oligosaccharide fraction of factory molasses caused z elongation. Analysis by gc showed three kestose isomers to be major components in factory molasses (Nurok 1975). Subsequently he prepared nearly pure kestoses and carried out crystallisation experiments in Ferrara in co-operation with Mantovani. The final results showed that kestose isomers in pure sucrose solution caused only moderate z elongation (Vaccari 1981).

The influence of inorganic components on the sucrose crystal shape has received less attention than that of poly- and oligosaccharides. Inorganic salts however also change the shape of sucrose crystals, and Mantovani (1974) reported the influence of potassium and barium. Potassium had a minor influence, but barium showed marked elongation in the b-direction, confirming earlier results of Vavrinecz (1965). Contrary to the influence of poly- and oligosaccharides that of salts cannot be explained by temporary or specific adsorption. The influence of inorganic components was not included in the work reported here.

EXPERIMENTAL

Crystallisation Rate Determinations

Initially crystals were attached to a holder and were grown in supersaturated solutions in a temperature controlled vessel similar to that used by Smythe (1967a) and by Broadfoot and Steindl (1980). The solution was stirred at various speeds but secondary crystallisation occurred frequently and it was decided to grow crystals under free fall. Eight glass tubes (130 x 30 mm with B 25 stoppers and holding about 50 ml solution) were attached to a motor driven wheel (ϕ 280 mm). The speed of rotation could be varied

electronically and could be monitored electromechanically. Most experiments were carried out at 20 rpm. The entire apparatus was placed in a temperature controlled oven at 60-61°C.

Sugar solutions were prepared by weighing first refinery boilings, impure factory products and water to give a sucrose/water (S/W) ratio of 3.075, i.e. a supersaturation of 1.06 for pure sucrose at 60.5°C based on data of Charles (1960). For most experiments the non-sucrose/water (NS/W) ratio was 0.1. Where molasses impurities were separated into oligosaccharides and polysaccharides 0.1 times the percentage of their presence in molasses was added. The components were dissolved at 70°C in a rotating flask and the clear solutions were cured for several hours in an oven held at 70°C. The cured solutions were transferred to the crystallisation tubes and equilibrated overnight at the working temperature (60-61°C).

A single crystal technique was used to measure crystal growth rate. Crystals were pre-grown from selected coffee crystals to about 75-100 mg at 1.04 degree of supersaturation. The crystals were weighed to 1 mg, tied to stainless steel wire formers using single filament nylon (ϕ 0.15 mm), pre-heated for 10-15 minutes and placed in the hot sugar solution at 60°C. After 5 hours the crystals were removed from the sugar solution, excess syrup was wiped off with soft tissue and the crystals were weighed again.

The crystal growth rate ($\text{kg.m}^{-2}.\text{sec}^{-1}$) was calculated according to

$$R = \frac{202,266 \times (M_f - M_i) \times 10^{-5}}{t \times (M_i^{2/3} + M_i^{1/3} \times M_f^{1/3} + M_f^{2/3})}$$

where M_i = initial mass (g)
 M_f = final mass (g)
 t = time (hrs)

Crystal Production for Shape Determination and Shape Measurement

For shape measurement a number of crystals were grown in the glass tubes described above. One drop of factory prepared ball mill slurry (0.5 mg - 1.5 mg of sucrose of about 4μ) was placed on the surface of the solution and the stoppered tubes were rotated on the

wheel without delay. In later experiments screened seed (100-125 μ) was used. The crystals were grown for 48 hours and the syrup mixture was poured into small perspex baskets (ϕ 30 mm x 55 mm) fitted with wire mesh (0.35 mm aperture), centrifuged at 3,000 rpm for 5 minutes, washed with methanol and recentrifuged. The washing step was repeated once more and the crystals were air dried.

The ratio of the crystal length in the b-axis direction (y) to that in the c-axis direction (z) was used as the shape parameter. Originally 200-250 crystals were crystallographically identified and the crystal lengths measured using a manual image analyser (Kontron MOP-Videoplan). Later it was shown experimentally that the same result was obtained by measuring fewer crystals (50 - 100) and expressing the shape as \bar{y}/\bar{z} . However, this gives no indication of the range of shapes encountered.

Crystals were dispersed in sucrose saturated glycerol for measurement of the shape in commercial boilings.

Isolation of Classes of Impurities

Final molasses originating from a refinery or raw sugar factory was diluted to 20°Bx and centrifuged at 10,000 rpm for 20 minutes at 15°C. The sediment was discarded. High molecular mass material was precipitated by adding 3.5 volumes of absolute ethanol to one volume of supernatant. After standing overnight the sediment was removed by centrifuging. The sediment was dissolved in warm water (400 cm³), reprecipitated with absolute ethanol (1,440 cm³) and kept overnight. The suspension was redissolved in warm water (300 cm³) and ultrafiltered (Amicon CH-4A concentrator, cut off 5,000 molecular mass) using a wash-out technique with 1,000 cm³ water to remove any low molecular mass material.

The retentate was freeze-dried (Fraction A). The combined supernatants from the alcohol precipitations and the ultrafiltrates were concentrated under vacuum at 40°C (Fraction B).

A carbon-Celite column was prepared by packing a glass column (50 x 1,000 mm) with a 1:1 slurry of Darco G-60 and Celite 545 in 50% ethanol. The column was washed with water (2-3 litres) before use. Water (50 cm³) was added to a portion of fraction B (ca. 100 g) and the solution was loaded onto the column at about 1 cm³ min⁻¹.

After collecting 150 cm³ the solvent was changed to 5% ethanol (2,100 cm³) and then to 50% ethanol. Fractions were monitored using thin layer chromatography (Schaffler and Morel du Boil 1972). The monosaccharides, sucrose and traces of trisaccharides eluted between 1,650 and 4,000 cm³. This eluate was concentrated under vacuum at 40°C (Fraction B₁). The oligomers eluted between 4,000 and 6,500 cm³. This eluate was concentrated in the same way (Fraction B₂). The isolation procedure is summarised schematically in figure 1.

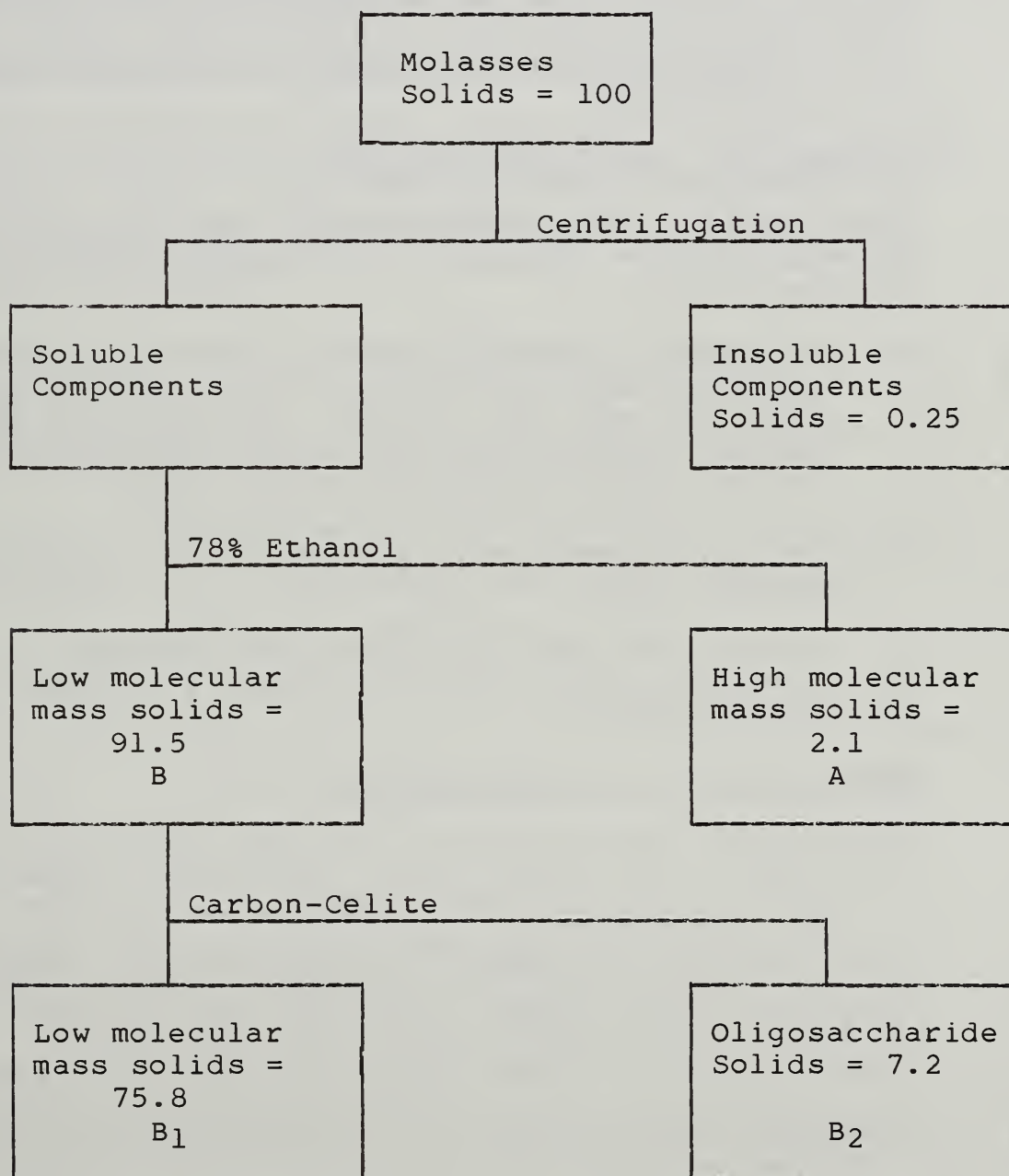


Figure 1.--Molasses fractionating scheme.

Fractionation of Oligosaccharides

The same carbon-Celite column was used for further separation.

One hundred grams of syrup B (molasses after removal of polysaccharides) was loaded on top of the column with 200 cm³ distilled water. The column was step-wise eluted with 5% ethanol (2 litres), 15% ethanol (2.25 litres), 50% ethanol (2.25 litres) and 75% ethanol.

The fractions were monitored by thin layer chromatography and combined as desired. The combined fractions were freeze-dried. A C-18 reverse phase hplc column was used for analysis and small-scale preparative separations.

Preparation of Oligosaccharides

Three classes of oligosaccharides were prepared and subjected to the isolation on a carbon-Celite column described above.

- (a) Iso-malto-oligomers were prepared by refluxing dextran (Sephadex T-150) (5 g) in 0.3N sulphuric acid (25 cm³) for 2 hours. The solution was cooled, neutralised with sodium carbonate and freeze-dried (Saska and Polack 1982).
- (b) Malto-oligomers were obtained from a commercial glucose syrup (DE 42).
- (c) Sucrose derived oligomers were prepared by reacting sucrose with invertase according to Gross (1962).

Hydrolysis of Oligosaccharides

Fraction B₂ and preparation (c) were hydrolysed using invertase. The sample (0.3 - 0.4 g) was dissolved in 0.2M sodium acetate-acetic acid buffer, pH 4.6 (3 cm³), and invertase (BDH 39020) (60 µl) was added. After reacting at ambient temperature for 2 hours the enzyme was inactivated by boiling the solution for 2 minutes. The cooled solution was neutralised (Amberlite IR 45 (OH)), filtered and freeze dried.

A control was prepared in a similar way but with the invertase omitted.

Chemical Analysis

All impure products and fractions were analysed for

sucrose using a gc technique (Schäffler and Morel du Boil 1984) and for water using a Karl Fischer method (MacGillivray and Nurok 1973). Low molecular mass fractions, column eluates, oligosaccharide preparations and hydrolysates were monitored or profiled using tlc (Schäffler and Morel du Boil 1972). The solvent polarity was varied by adjusting the ethanol component (28 to 38%) and the development time varied between 1 h and 48 h depending on the application (i.e. fast monitoring or detailed resolution).

RESULTS AND DISCUSSION

Crystal Shape and Growth Rate Determination

Table 1 shows the crystal shape measurements for different boilings in various refineries. It shows that crystal shape varies from refinery to refinery and that as impurity levels increase the crystals show increased \bar{z} elongation. Recovery boilings show the lowest \bar{y}/\bar{z} ratio.

Table 1.--Crystal shape (\bar{y}/\bar{z}) for different boilings at several refineries

Boiling number								
Refinery	Refinery				Recovery			
	1	2	3	4	1	2	3	
A Dec.	1.05	0.90	0.55	0.55	0.25	0.25	0.25	
Aug.	1.30	1.10	1.00	0.55				
B May	1.55	1.35	0.90	0.80				
Sept.	1.45	1.20	0.90	0.65				
C Oct.	1.45	1.15	1.20	0.60				
Dec.	1.50	1.50	1.20	-				
D Aug.	1.55	1.40	1.25	0.85				
Nov.	1.35	1.15	0.85	-				
E Aug.	1.35	1.15	1.10	-				
F Aug.	1.60	1.40	1.55	-				
Nov.	1.45	1.25	1.40	-				

Initially most experiments were carried out with exhaust molasses from recovery boilings from a refinery as this showed the highest concentration of impurities causing z elongation.

Under controlled laboratory conditions, increasing levels of this molasses were added to pure sucrose solutions. The ratio S/W was kept at 3.075 and the NS/W ratio increased from 0.05 to 0.60. The results are shown in figure 2.

In addition to shape measurements crystallisation rate determinations were done. Habit modifiers retard growth in specific directions which results in a decrease in overall growth rate. Broadfoot and Steindl (1980) indicated that the solubility of sucrose affects the supersaturation by about 5% in the NS/W ratio used in this investigation. As the growth rate measurement is not extremely accurate constant supersaturation has been assumed for a particular molasses in this NS/W range. Rates were compared at constant S/W ratios. This means that the rates include a slight solubility influence as well as a habit influence in addition to a temperature effect. In all the experiments an air thermostat was used instead of a water bath which had the effect that temperatures dropped 5 to 6°C below the set point of 60°C. However they recovered in less than 20 minutes to within 1°C of this set point. The final recovery

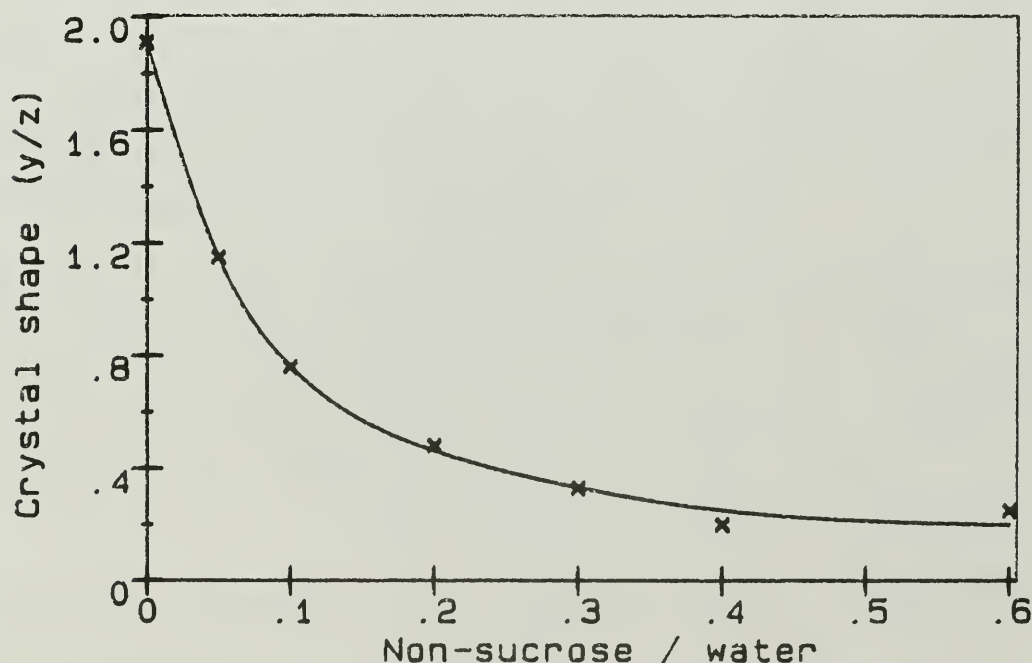


Figure 2.--Effect of impurity concentration on crystal shape.

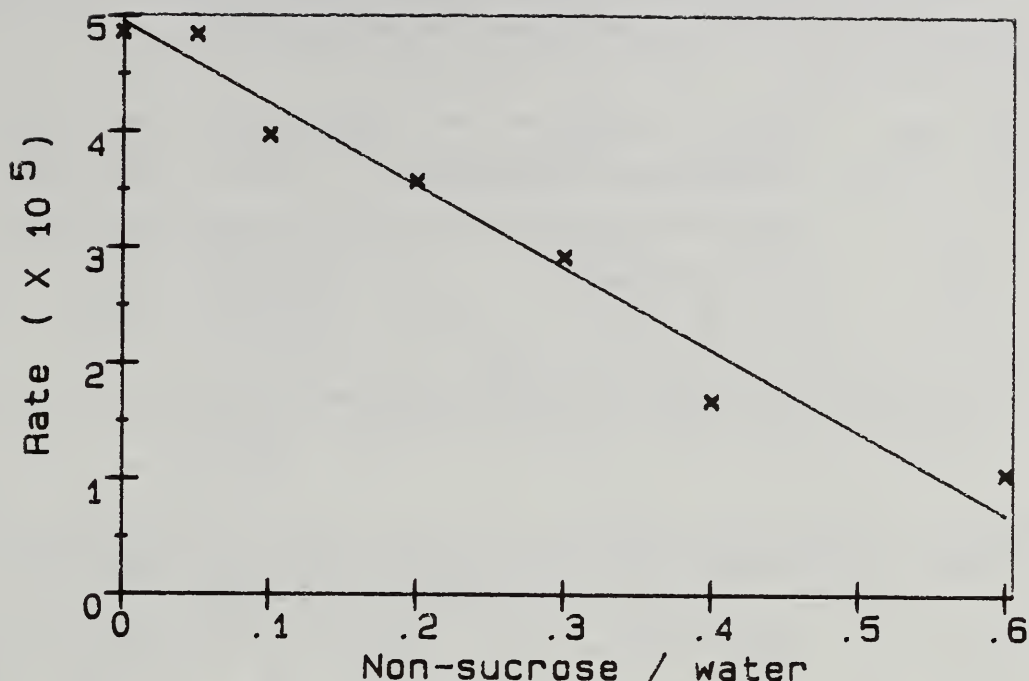


Figure 3.--Effect of impurity concentration on crystal growth rate at 60°C and S/W = 3.075.

took 1 to 1½ hours due to the poor heat transfer of an air thermostat. Two experiments were not exactly reproducible for this reason and the growth rate values show an appreciable variation with a tendency to overestimation. The advantage of the crystalliser used is that it allows comparison of eight solutions under identical conditions.

Figure 3 illustrates the decrease in crystallisation rate as the impurities increase and comparison with figure 2 shows that the crystal shape alters simultaneously.

In the experiments using fractions of the original molasses the recovery of sucrose was 90% after fractionation and 80% for non-sucrose indicating physical and selective losses. However when fractions were recombined in proportion to their actual yields it was evident that the elongating properties remained unaltered (table 2). The rsd's represent the variation of shape within a batch of crystals. The average batch to batch mean for molasses impurities at NS/W = 0.1 was 0.76 ± 0.04 (rsd = 5%) for seventeen batches with a range of 0.71 to 0.85.

The rate and shape measurements obtained with the individual fractions when added at concentrations present in the original molasses are summarised in tables 3 and 4.

Table 2.--Crystal shape ($\overline{y/z}$) in reconstituted molasses (NS/W equivalent to 0.1 impurities in molasses)

Combined fractions	y/z	rsd (%)
Molasses	0.75	30
A + B	0.75	33
A + B ₁ + B ₂	0.75	32

Table 3.--Comparison of growth rates in various molasses fractions

Fraction	Rate (kg.m ⁻² .sec ⁻¹) (X 10 ⁵)	
	Mean	sd
Sucrose	5.7	0.8
Molasses	3.1	0.3
A	6.5	0.4
B	4.2	0.3
B ₁	5.1	0.8
B ₂	2.2	0.3

Table 4.--Comparison of crystal shape in various molasses fractions

Fraction	$\overline{y/z}$		
	Mean	sd	rsd (%)
Sucrose	1.85	0.50	27
Molasses	0.75	0.22	29
A	1.95	0.61	31
B	0.80	0.27	34
B ₁	1.70	0.47	28
B ₂	0.90	0.31	34

Table 5.--Comparison of elongation with molasses polysaccharide and with standard dextran

Compound	NS/W*	\bar{y}/\bar{z}	rsd (%)
Fraction A	0.007	1.60	28
Fraction A	0.035	1.55	23
Dextran (T-40)	0.033	1.70	36

* Fraction A at NS/W = 0.007 is equivalent to using unfractionated molasses at NS/W = 0.100.

The rate-retarding and habit-modifying properties were shown to be in the oligosaccharide fraction. Little effect was caused by the polysaccharide fraction. The polysaccharide fraction, which contained 50% dextran as determined by the Roberts (1983) method decreased the \bar{y}/\bar{z} ratio to 1.55 when added at five times the concentration in which it was present in molasses. This agrees with the slight decrease in \bar{y}/\bar{z} ratio found for pure dextran (table 5).

Raw Sugar Factory Impurities

Similar experiments were carried out with molasses from raw sugar factories experiencing crystal deformation. The separation of these impurities, however, was not carried further than the precipitation with 78% ethanol.

The results are shown in table 6 for the poly-

Table 6.--Polysaccharides and crystal shape (\bar{y}/\bar{z})

Factory	Polysacs. (% NS)	Polysacs./W @ NS/W equivalent to		\bar{y}/\bar{z}
		0.1	0.5	
Mill	A	6.1		1.30
	B	6.1	0.031	1.45
	C	6.7	0.034	1.60
Refinery	D	9.3	0.009	1.55
	E	6.6	0.007	1.60
	F	5.9	0.006	1.50
	G	6.2	0.006	1.60
	H	7.0	0.007	1.95
	H		0.035	1.55

saccharide fraction and in table 7 for the oligo-saccharide fraction.

In order to obtain \bar{y}/\bar{z} ratios of about 0.8 to 1.0 in laboratory experiments with unfractionated molasses the purity is about 86 compared to 97 for refinery products. At these lower purities the polysaccharide concentration is about five times that for refinery solutions and for this reason the polysaccharides are making a noticeable contribution to \bar{y}/\bar{z} ratio reduction.

Oligosaccharide concentrations in raw sugar factory products relative to total non-sucrose are about one-fifth of those in refineries. Since five times more non-sucrose was used for raw sugar factory experiments, the crystals were actually grown in solutions containing similar oligosaccharide concentrations (table 7).

Table 7 also shows that, for the same concentration, oligosaccharides originating from refineries cause slightly lower \bar{y}/\bar{z} sucrose ratios than those from raw factories. This could be due to a difference in the type of oligosaccharides present.

These trends are also illustrated in figure 4 where crystal shape (\bar{y}/\bar{z}) has been plotted against oligosaccharide concentration. Although total non-sucrose is higher for the mill, crystal elongation is similar for both refinery and mill at similar oligosaccharide/water ratios.

Table 7.--'Oligosaccharides' and crystal shape (\bar{y}/\bar{z})

Factory		Oligosacs. (% NS)	Oligosacs./W @ NS/W equivalent to		\bar{y}/\bar{z}
			0.1	0.5	
Mill	A	2.8		0.014	1.20
	B	3.0		0.015	1.20
	C	1.3		0.007	1.20
Refinery	D	-	N/A		0.60
	E	11.3	0.011		0.85
	F	-	0.013		0.80
	G	12.5	N/A		0.80
	H	11.5	0.012		0.80

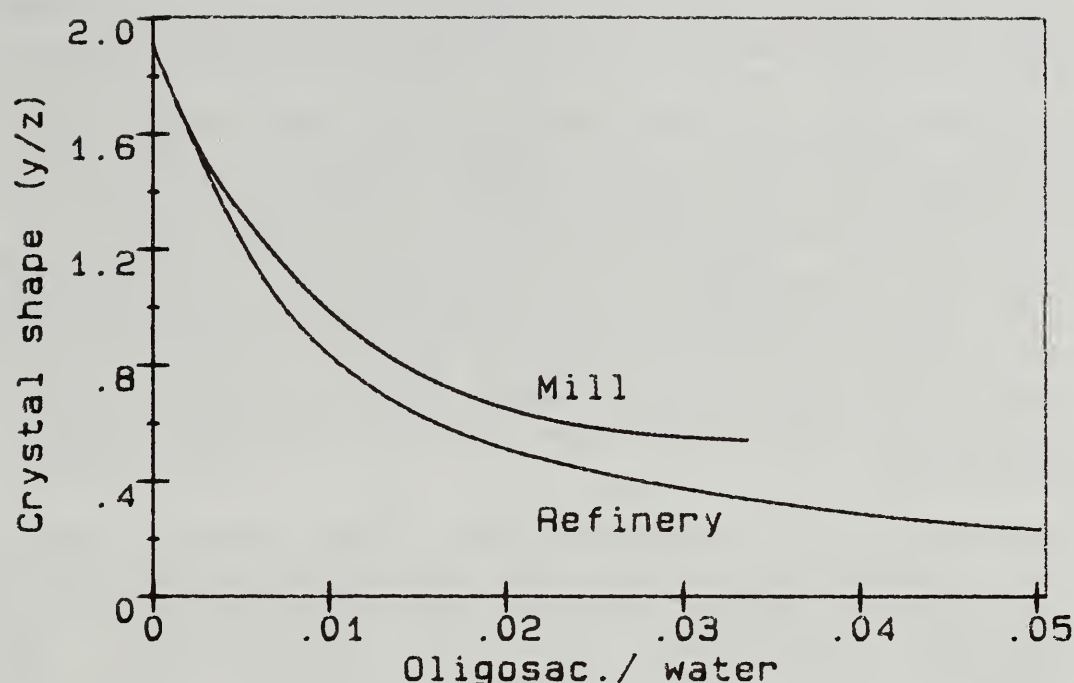


Figure 4.--Effect of oligosaccharide concentration on crystal shape.

The possible components in the oligosaccharide fraction are iso-maltose homologues (hydrolysis products of dextrans), maltose homologues (hydrolysis products of amylose) and fructosyl-sucrose or glucosyl-sucrose oligomers. Crude extracts of these classes were added to sucrose in the same proportion as Fraction B₂. The results are listed in table 8.

Table 8.--Effect of oligosaccharide classes on crystal elongation

Oligosaccharide	NS/W*	$\overline{y/z}$	
		Mean	rsd (%)
Sucrose	-	2.00	29
Fraction B ₂	0.013	0.90	41
Isomalto-series	0.014	1.75	33
Malto-series	0.014	1.80	30
F-S and G-S series**	0.014	2.05	32

* Fraction B₂ at NS/W = 0.013 is equivalent to using unfractionated molasses at NS/W = 0.100.

** F-S = fructosyl-sucroses, G-S = glucosyl-sucroses.

Addition of maltose or iso-maltose oligomers showed little y/z decrease, while oligosaccharides prepared from sucrose (mainly 6- and neo-kestose) caused slight y elongation. This anomaly might have been caused by other impurities in the preparation as 6- and neo-kestose are reported to cause a slight z elongation. Only fraction B_2 showed substantial y/z ratio depression. Crystals grown in the presence of this fraction were small and conglomerated. Moller (1954), Pot (1983) and Kuijvenhoven (1983) have indicated that conglomeration occurs mainly below a critical particle size. At a slow growth rate crystals remain for a long time below this size causing increased conglomeration.

Hydrolysis of fraction B_2 and of the kestose preparation completely removed the elongating properties so that normal shaped crystals were obtained when sucrose solutions were spiked with these hydrolysates. The control (B_2 + buffer) showed a slightly higher y/z ratio than the original fraction (B_2) (table 9), but this is probably within the experimental error. From this it can be concluded that fructosyl-oligosaccharides were probably responsible for the z elongation in refinery massecuites.

Results from further separation of the oligosaccharide fraction are shown in table 10.

The group of lower molecular mass oligosaccharides ($B_{2.1}$) shows the highest effect on z elongation. Neo-kestose was isolated from this group and was shown to cause only moderate z elongation at the concentration present in refinery molasses. This is in agreement with earlier work and other published results.

Table 9.--Influence of invertase hydrolysis on elongating properties

Preparation	$\overline{y/z}$	
	Mean	rsd(%)
F-S and G-S series	2.25	38
(F-S and G-S) after hydrolysis	1.80	30
B_2	0.90	34
Control (B_2 + buffer)	1.10	34
B_2 after hydrolysis	1.95	29

Table 10.--Crystal elongating properties of oligo-saccharide sub-fractions isolated from refinery molasses

Fractionation Technique			% Mol. Solids	\bar{y}/z
Ethanol Precipitation	Ethanol Elution from Carbon	Reverse Phase C-18		
Fraction B			95	0.80
	B ₁ (5% EtOH)		79	1.60
	B _{2.1} (15% EtOH)		5.6	1.30
		B _{2.1.1}	4.8	1.45
		B _{2.1.2}	0.8	1.75
	B _{2.2} (50% EtOH)		2	1.65

Fraction B_{2.1} was analysed by hplc on a C-18 reverse phase column. The chromatogram is shown in figure 5 together with tentative peak assignment.

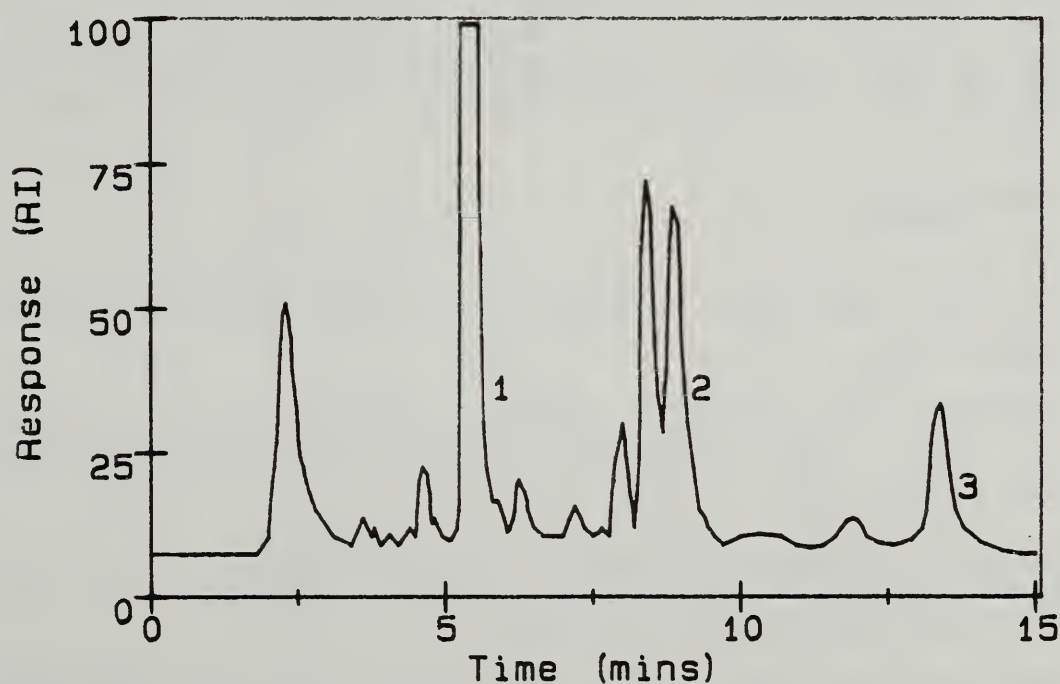


Figure 5.--Hplc chromatogram of fraction B_{2.1}.
Tentative identification: 1=sucrose; 2=l-kestose;
3=neo-kestose.

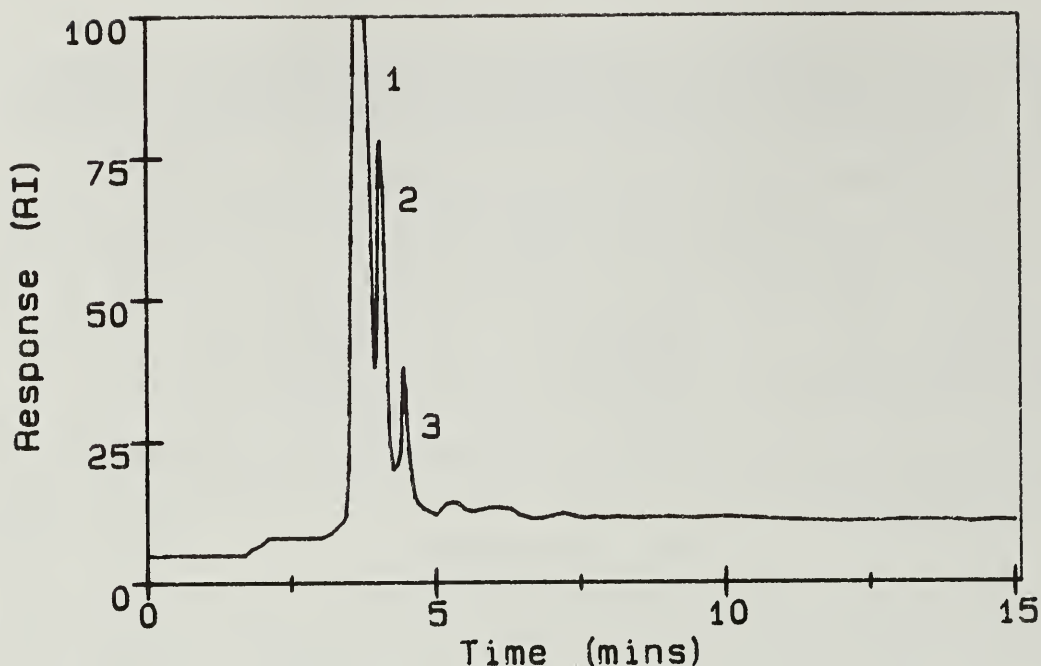


Figure 6.--Hplc chromatogram of fraction B_{2.1} after hydrolysis with invertase. Tentative identification: 1=fructose and glucose; 2=maltose and iso-maltose; 3=iso-maltose.

Figure 6 shows an hplc chromatogram of the same fraction after hydrolysis with invertase. Apart from a few minor peaks all the original oligosaccharides have been hydrolysed. Besides the two major monosaccharides, maltose and iso-maltose have been tentatively identified, both by hplc and tlc profiling, indicating the presence of glucosyl-sucrose oligosaccharides in fraction B_{2.1}.

CONCLUSION

Oligosaccharides caused most of the c-axis elongation. This was the case in both refineries and raw sugar factories.

However, in raw sugar factories some of the elongation was due to the presence of higher concentrations of polysaccharides.

Maltose oligomers, iso-maltose oligomers and a kestose mixture did not appear to be major contributing factors in crystal deformation.

Hydrolysis by invertase completely removed elongating properties from the oligosaccharide fractions under investigation.

Hplc and tlc profiling tentatively indicated that the oligosaccharide fraction contains at least two fructosyl-sucroses and at least two glucosyl-sucroses.

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DISCUSSION

Andrew VanHook, College of the Holy Cross - Have you used HPLC on the preparative scale to separate these components, rather than only as an analytical tool?

Bruijn - No. We have used carbon-celite columns and reverse phase columns. Reverse phase was used on a semi-preparative scale; we collected sufficient material to use in crystallization studies. We plan more preparative work because now one's aim is to isolate and identify these compounds.

Margaret A. Clarke, S.P.R.I. - What is the upper molecular weight limit, or degree of polymerization, of your oligosaccharide fractions?

Bruijn - My co-author has warned me not to speculate too much on this. However, there are strong indications that this compound is not a fructosyl-sucrose, but rather a glucosyl-sucrose or a maltosyl-sucrose. Invertase enzyme will break a glucosyl-sucrose bond to form either maltose or isomaltose; we obtained both of these. These results are not included in the paper, but work is continuing.

Clarke - Is fructose one of the terminal groups of the oligosaccharide? That's rather unusual.

Bruijn - Fructose is part of the sucrose molecule in the oligosaccharide. As we do not know the exact structure, this fructose part could be terminal or not depending at which side the glucose or maltose is attached.

Clarke - Do you think this could have been made through a glucosyl- or fructosyl-transferase system?

Bruijn - It's probably a compound native to the cane plant that comes in to the factory in the cane rather than being made in process. We see the elongation factors increase at certain periods of the year although the process remains the same, so I think they are in the cane. But that will require another investigation.

Dennis Martin, Tate and Lyle - Could you give us a little further indication of the most likely structures of the elongating oligosaccharides?

Bruijn - Yes, they are likely to be glucose-sucrose or maltose-sucrose compounds. Our next step is to isolate these discretely, either by preparative HPLC, or by cellulose column chromatography, because we want to isolate a reasonable quantity. Then we will determine the structure. Another approach is to guess at the structure and synthesize possible compounds, by various enzymes. A third possibility is the use of enzymes--there are various enzymes claimed by their manufacturers to eliminate elongation factors--so far, we've used only invertase.

CRYSTALLIZATION STUDIES IN LOW GRADE SYRUP

Raymond E. Dickey and Joseph F. Dowling
Refined Sugars Inc.

Robert M. Morton
Tate & Lyle Group Research & Development

INTRODUCTION

In an effort to determine the effect of crystal number on sucrose exhaustion from low grade syrup, it was necessary to find a system through which individual crystal volumes could be measured both quickly and accurately.

Refined Sugars Inc. in conjunction with Tate & Lyle Group Research & Development, Reading, U.K., developed a method for crystal measurement using a VIDS system with ancillary D.O.S. software.

In addition to the crystal number, the effects of several other parameters, such as "g", massecuite purity, seed volume, and axial ratio were examined with respect to their effect on sucrose exhaustion.

DESCRIPTION OF THE EQUIPMENT

The VIDS standard software runs on the Apple II microcomputer equipped with at least one disc drive and an Apple Graphics tablet with a printer. Each has an interface in one of the expansion slots numbered 1-6 within.

The microcomputer contains a 6502 processor with a main memory and speaker which is used to produce beep sounds.

A video camera and a 12-inch green video monitor are interfaced with the microcomputer so that images from the slide which contains the crystal slurry can be transferred to the monitor screen for measurement.

The D.O.S. (Disk Operating System) contains the program as well as the files for size ranges to which the individual crystals will be compared after measurement.

SLIDE PREPARATION

The slides for measuring the crystal size of the massecuite were prepared by heating the massecuite in water at about 70° C until the massecuite assumed fluidity. The massecuite was then mixed with invert syrup at about 76% solids. Ordinarily, a 50/50 V/V mixture will insure a free-flowing mixture so that the slide will allow good differentiation between individual crystals and therefore facilitate the measurement.

MEASUREMENT OF THE CRYSTALS

Since the crystals in the massecuite were not normally measured immediately after the pan was dropped, sizings were determined for different time differentials, i.e., crystals were measured immediately after the pan was boiled and the same sample was held for 24 hours and measured again. The variation in crystal sizes was not significant for the two time periods.

Crystal measurement was initiated by placing a small drop of the massecuite/invert slurry on the slide, and a thin glass coverlet was placed in contact with the droplet and gently pressed down so as to effect a thin film. The slide was then placed in position on the moveable stage of the microscope so that light from a high intensity lamp passed through it from below.

The images of the crystals on the slide were transferred to the television monitor and could be measured by interfacing the Apple Graphics tablet and indicator pen. The program initially will display:

VIDS 4 DOT PARTICLE SIZING

The program will then request a title, which can be entered from the keyboard if desired. This is followed by a request for date and calibration change if desired. If no calibration change is needed, the sequence is continued by pressing the space bar on the keyboard. The computer will then request a sample entry, which can be typed in from the keyboard. Once the particular sample has been entered, a rectangle will appear on the screen containing a cross section of the crystals to be measured within its borders.

When the indicator pen was placed on the graphics tablet within the borders of the rectangle, a small cross could be seen, which indicated the relative position of the pen tip to the crystal images on the screen. By manually moving the pen through the rectangle, it was possible (with some acquired dexterity) to bring the illuminated cross in contact with the edge of a particular crystal. The pen was then pressed down gently, and a dot was transposed to the screen corresponding to the edge of the crystal. The pen was then moved along the length of the crystal to the other edge, and the procedure was repeated.

The distance between the two points was transferred to the main memory for comparison to the size ranges stored in the long axis file.

The pen was then moved to the adjacent edge of the crystal and once again gently pressed. Finally, the pen was moved across the width (or short axis) of the crystal, and the pen was pressed once more. When the crystal was surrounded by the four dots, a beep sound was heard indicating that the crystal measurement was complete.

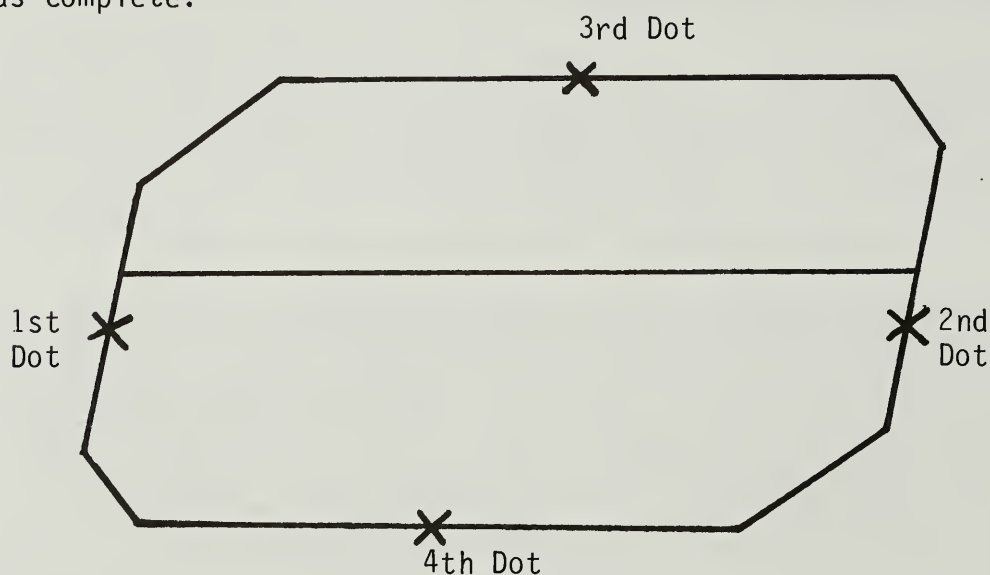


Figure 1

The measurements were repeated for all of the crystals within the rectangular border, and the slide was then moved so that another cross-section appeared on the screen. The Apple computer was programmed to measure 200 crystals and then to proceed automatically into a crystal-sizing mode.

It should be stated that when the crystals were measured within the border, some subjective judgment was required as to which crystals should be included.

If the field contained a small number of very tiny crystals in the background, these would be ignored since they were considered outside of the normal distribution. However, if a significant number of tiny crystals were present with the larger ones, the crystal distribution was considered bi-modal, and so all crystals would be included in the measurement.

Since as stated above, a certain degree of judgment was required to measure crystals within a field, it was decided to have four different chemists measure the same slide. No significant differences between the measurements were found.

SIZING OF THE CRYSTALS

The VIDS system included a "Disk II" disk storage system, which consisted of a floppy disk which was inserted into a compact cabinet. The floppy disk contained the program, the current calibration, and files, which in turn contained size ranges for length, width, volume, and axial ratio, i.e., b axis/c axis.

It should be noted here that when the crystals were measured with the indicator pen, it was only possible to measure in two dimensions: length and width. Therefore, when computing a sizing for volume, the program made the assumption that the width and thickness of the crystal were identical.

$$\therefore \text{Volume mm}^3 = l \times w \times w$$

Also the assumption was made that the b axis was always smaller than the c axis, and consequently, the axial ratio would always be less than unity.

After 200 crystals were measured, the program automatically displayed a menu which would allow sizing calculations to be performed by entering letter "s". The program would then call for particular file names which would, in turn, give ranges for short axis length, long axis length, axial ratio, and volume.

By calling up a particular file and giving the corresponding instruction, the computer performed a sizing for each of the crystals in memory and printed out a distribution of the size ranges within the parameters of the file as well as an average size of the crystals measured. The size distribution ranges could be divided in any way, up to a maximum of 30 distribution bands. A typical data sheet showing distribution bands and average size for crystal volume is shown in Figure 2.

ANALYSIS OF THE MASSECUITE

To determine what the variables were in a particular massecuite, certain analyses were necessary. Prior to measurement of the crystals, the massecuite was analyzed for solids and apparent purity. The massecuite was then passed through a vacuum filter containing a screen of 50 mesh to remove the crystals. This was followed by a determination of purity on the molasses filtrate.

By applying the SJM formula, the percent crystals in the total massecuite could be determined. By knowing the total volume of the "C" pan, the pounds of crystals could be calculated. The remelt pan for boiling "C" strikes was estimated to contain 39.64m^3 of massecuite when filled to capacity. Since the

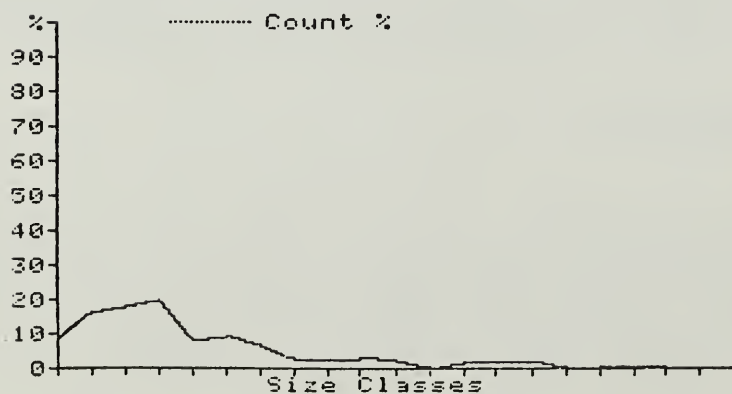
Figure 2--Computer Printout of Crystal Volume Distribution

C-MASS 507-2

6 3 96

Volume (millimeters ³)	COUNT	COUNT %
0 - 1E-03	17	8.5
1E-03 - 2E-03	32	16
2E-03 - 3E-03	36	18
3E-03 - 4E-03	40	20
4E-03 - 5E-03	16	8
5E-03 - 6E-03	18	9
6E-03 - 7E-03	12	6
7E-03 - 8E-03	4	2
8E-03 - 9E-03	5	2.5
9E-03 - .01	6	3
.01 - .011	3	1.5
.011 - .012	0	0
.012 - .013	3	1.5
.013 - .014	3	1.5
.014 - .015	3	1.5
.015 - .016	0	0
.016 - .017	1	.5
.017 - .018	1	.5
.018 - .019	0	0
.019 - .02	0	0
.02 -	0	0

Mean: 4.241E-03



specific gravity may be stated as 1.5 tons/m^3 ,

$$\therefore m^3 \text{ "C" Mass} \times \% \text{ Crystals} \times 1.5 = \text{Crystal Tons}$$

$$\text{Crystal Tons} \times 2240 = \text{Crystal Pounds}$$

As stated above, a full capacity pan contains 39.64m^3 of massecuite,

$$\therefore 39.64 \times \% \text{ Crystals} = \text{Total Crystal Volume}$$

Now from the measurement data of 200 crystals, the average volume of a crystal (mm^3) was calculated. Therefore, it was now possible to calculate the total number of crystals per strike.

$$\frac{\text{Total Crystal Volume}}{\text{Single Crystal Volume}} = \text{Total Crystal Number}$$

DATA EXAMINATION

The analysis of both the massecuite and corresponding molasses samples, as well as the crystal measurement of the massecuite, made it possible to determine the effect of different variables on the molasses exhaustion. The variables examined were:

1. Volume of Seed Added
2. Massecuite Purity (P)
3. Molasses Purity
4. Percent Crystal Solids in Strike
5. Total Weight of Crystals in Strike
6. Total Massecuite Solids in Strike
7. Total Number of Crystals in Strike (N)
8. "g" (Invert/Non-Sugars) in Molasses
9. Average Crystal Axis (Long)
10. Average Crystal Axis (Short)
11. Average Crystal Volume
12. Average Crystal Axial Ratio (b/c)

By the use of multiple regression analysis on a total of 370 samples, it was possible to determine the effect of the aforementioned variables against the molasses exhaustion. The latter was measured in terms of the difference between actual and ideal total sugars and was calculated according to the following formula:

$$\text{Plus Ideal} = \text{Total Sugars} - \frac{100 (5 + 3g)}{3 (3 + g)} \quad (1)$$

For the purposes of this paper, we have subtracted a constant value from the calculated plus ideal. This allows us to illustrate the effects on molasses exhaustion without specifying detailed process data.

The multiple regression analysis was performed by Tate & Lyle Group Research & Development at Reading, U.K., using commercially available statistics programs.

In the initial stage of the study, three of the twelve variables examined were shown to have a significant effect on molasses exhaustion. These variables were "g", massecuite purity, and crystal number.

As the study continued, the effect of crystal number became minimal. This can be explained by the fact that in the initial stage, small amounts of powdered sugar were used to seed the strike. As the volume of powdered sugar was increased above 500 cc, the effect of crystal number on sucrose exhaustion decreased markedly. An explanation of this will be offered later in the paper.

Multiple regression analysis of the later strikes with seed in the 500-1000 cc range gave the following regression equation (250 strikes):

$$\text{Plus Ideal} = 3.66g + 0.15P - K \quad (2)$$

where "g" = Invert/Non-Sugars
P = Massecuite Purity
K = Constant

Given the normal range over which "g" and P vary, it was clear that "g" has the largest overall effect.

This equation has been used to correct for the effect of "g" and P before examining plus ideal trends with other variables.

ANALYSIS OF VARIABLES

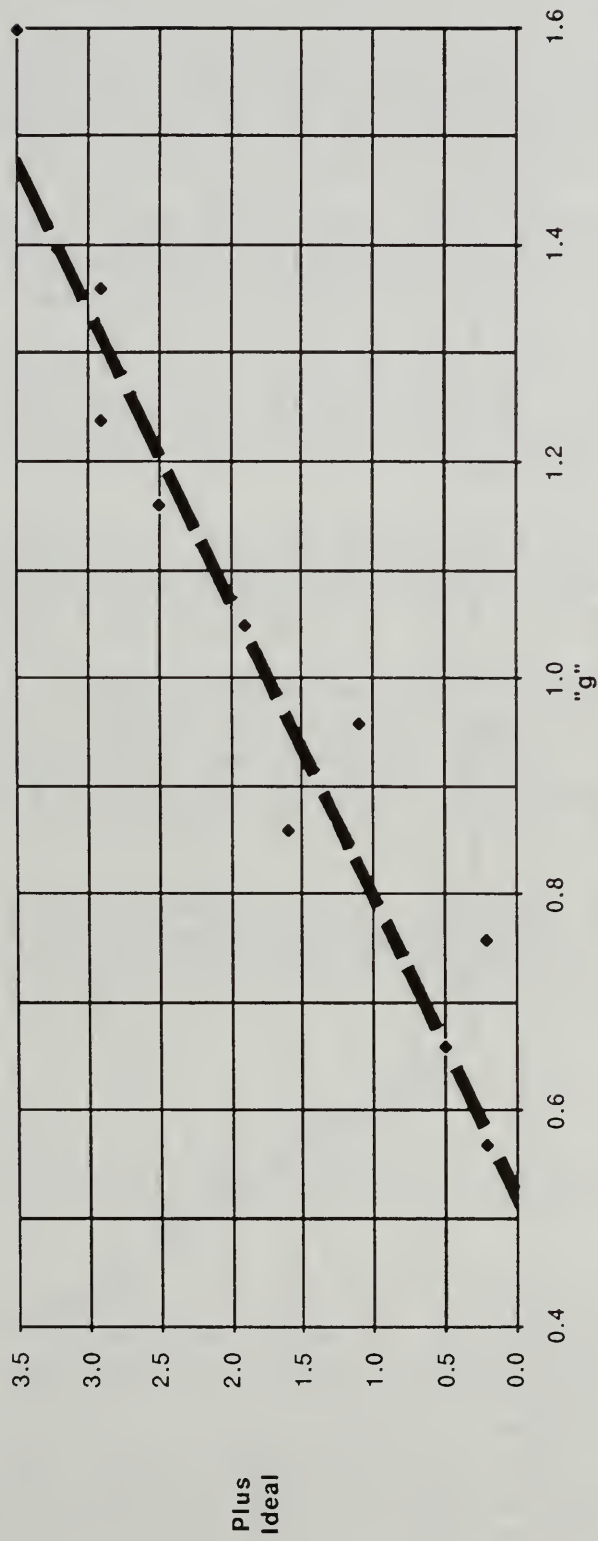
Effect of "g"

Graph I shows the trend of increasing "g" to molasses "plus ideal" performance. The "plus ideal" is calculated by subtracting a constant from the actual ideal. Each point on the graph represents the average of a number of individual data points (20-60).

Examination of the graph shows that as "g" increased, the points over ideal showed a corresponding increase. The graph relates relative ideal increases without taking into account the effect of massecuite purity.

The slope of the line shows that a doubling of the "g" value from 0.6 to 1.2 would cause an increase of approximately 2.5 units above the ideal value for sucrose exhaustion.

Graph 1
Graph Showing the Effect of Plus Ideal to
Increasing "g"



Note: Each point represents 20-60 strikes

Effect of Crystal Number

Examination of crystal number, as shown in Graph II, demonstrates that in order to increase sucrose exhaustion (or reduce points over ideal), it is necessary to increase the crystal number to a certain minimum.

The plot of data points, from our experience, shows that the minimum number of crystals required for maximum sucrose exhaustion is in the area of $10 \times 10^{+11}$.

Increase in crystal number above this value did not appear to have a marked increase in the exhaustion of sucrose, which is evidenced by a change in the slope of the trend line.

This change in trend might be explained by the fact that with a small crystal number, the total surface area available for sucrose deposition might not be sufficiently large, and so a certain minimum is required.

If sucrose exhaustion may therefore be said to increase with increasing surface area, it might be argued that performance should continue to improve with increasing crystal number.

The fact that this did not occur throughout might be explained by the fact that as the number of crystals became increasingly large, the individual crystal volume became increasingly small, i.e., as the number approaches infinity, the volume approaches zero.

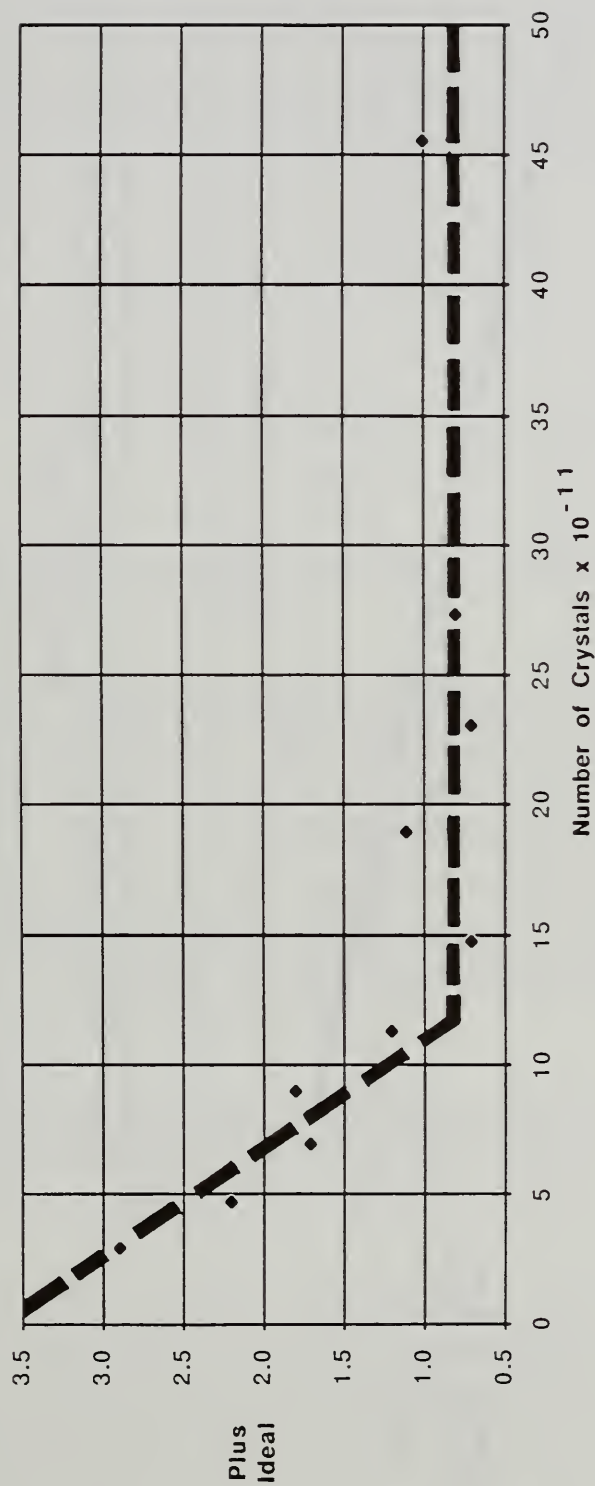
Therefore, very large numbers of crystals would have a volume sufficiently small to pass through centrifugal screens with consequent loss to molasses and the negation of any beneficial effect of large numbers.

Effect of Average Crystal Volume

As would be expected, the crystal volume has a relationship similar to crystal number with sucrose exhaustion or ideal performance.

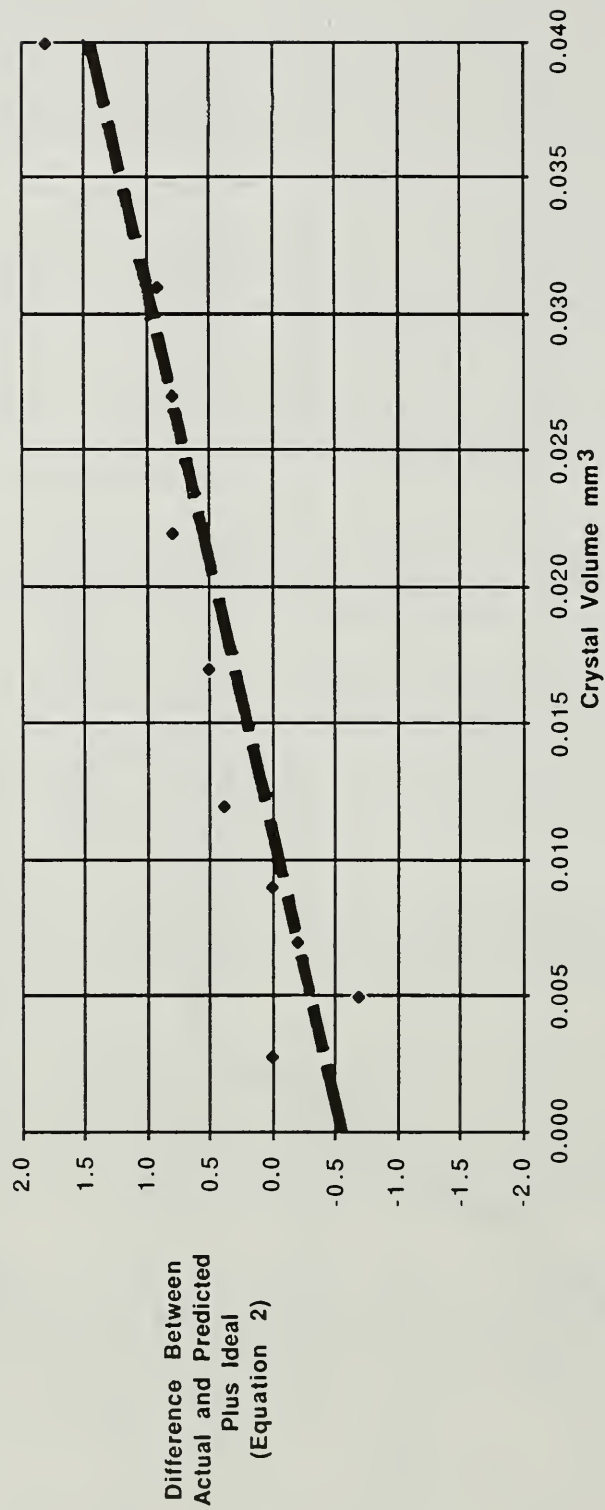
The theoretical ideal is the predicted ideal from the regression equation which compensates for "g" and mass purity effect (equation 2). The difference between actual and theoretical is shown in Graph III, where groups of data points are plotted in order of ascending crystal volume along the x axis. In Graph III the central or "zero" line represents the average "plus ideal" predicted by the regression equation, and differences above or below the line represent the differences of the actual "plus ideal" from it.

Graph II
Graph Showing the Relationship of Plus Ideal to the
Number of Crystals



Note: Each point represents 20-60 strikes

Graph III
Graph Showing Increasing Crystal Volume to
"Ideal Difference"



Note: Each point represents 20-60 strikes

There is obviously a fundamental relationship between the crystal volume and the crystal number. Graphs II and III are thus similar expressions of the same phenomenon, namely, the beneficial effect of a larger total crystal surface area.

Effect of Powdered Sugar Volume

The volume of powdered sugar added to grain strikes was varied in increments of 100 cc, from 100 cc to 1000 cc. The seed was prepared by adding 12 gallons of isopropyl alcohol to 60 pounds of granulated sugar and then grinding in a ball mill for a period of 24 hours. The resulting slurry was then measured in the appropriate amount using a graduated cylinder.

As a quantitative control, 100 cc of seed was drawn from each batch, filtered, and allowed to air dry. The weight of dried powdered sugar averaged 55 gm per 100 cc of slurry.

Graph IV shows the relationship of the seed of powdered sugar volume to the ideal performance. The x axis of the graph shows the volume of seed used, and the y axis represents the difference between actual "plus ideal" and theoretical "plus ideal" calculated from the regression formula.

Examination of the graph shows that for low volumes of seed--100, 200, 300 cc--the actual "plus ideal" value was higher than the formula prediction. Additions between 400-700 cc showed improved performance with the difference close to zero. Increasing further to 800 and 1000 cc shows additional improvement.

Since 1000 cc of seed was the largest amount added, it would appear that further studies are needed to see whether larger amounts of seed would further improve sucrose exhaustion.

Effect of Massequite Purity

Graph V represents a plot of average data points which relate the increase of massequite purity to sucrose exhaustion performance ("plus ideal"). The trend line shows that the points above ideal tend to increase with increasing massequite purity.

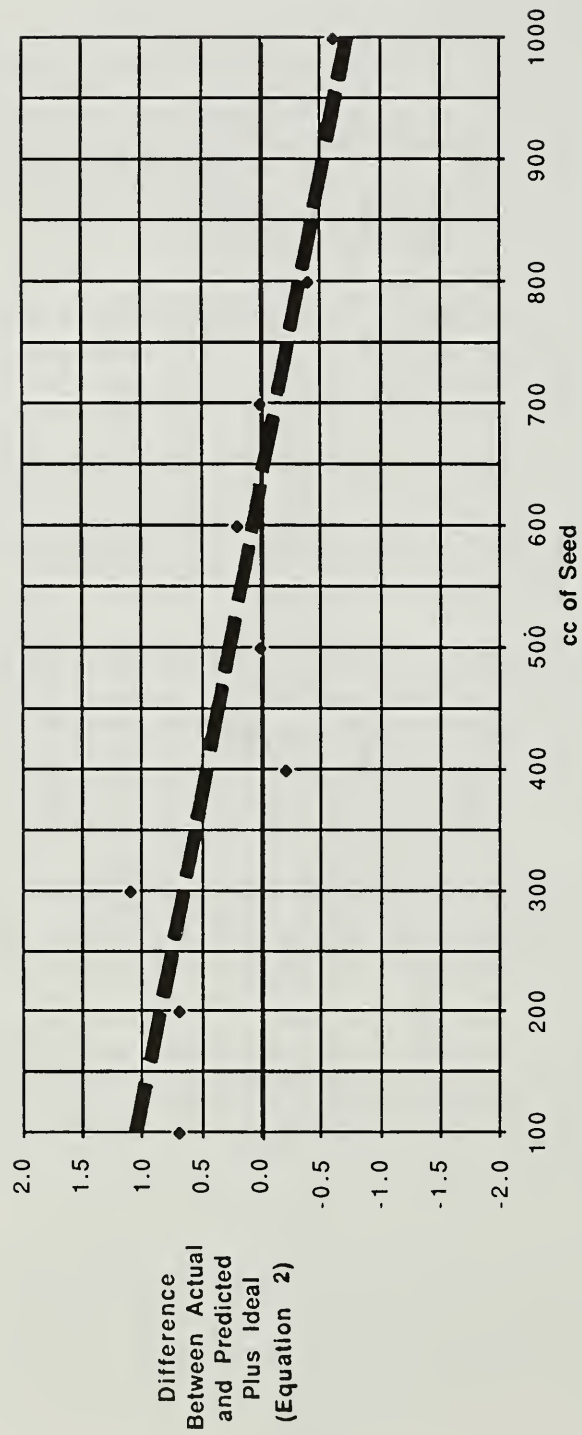
A comparison of the actual "plus ideal" minus theoretical "plus ideal" vs. mass purity is shown in Graph VI. The theoretical ideal was calculated using the ideal regression equation, fixing mass purity at 60 and using actual "g" values. This then eliminates the effect of "g" and shows better the correlation between purity and ideal.

Effect of Axial Ratio

It is known that dextran causes crystal elongation along the c axis (Sutherland, D. N., and Paton, N. H., Dextran and Crystal

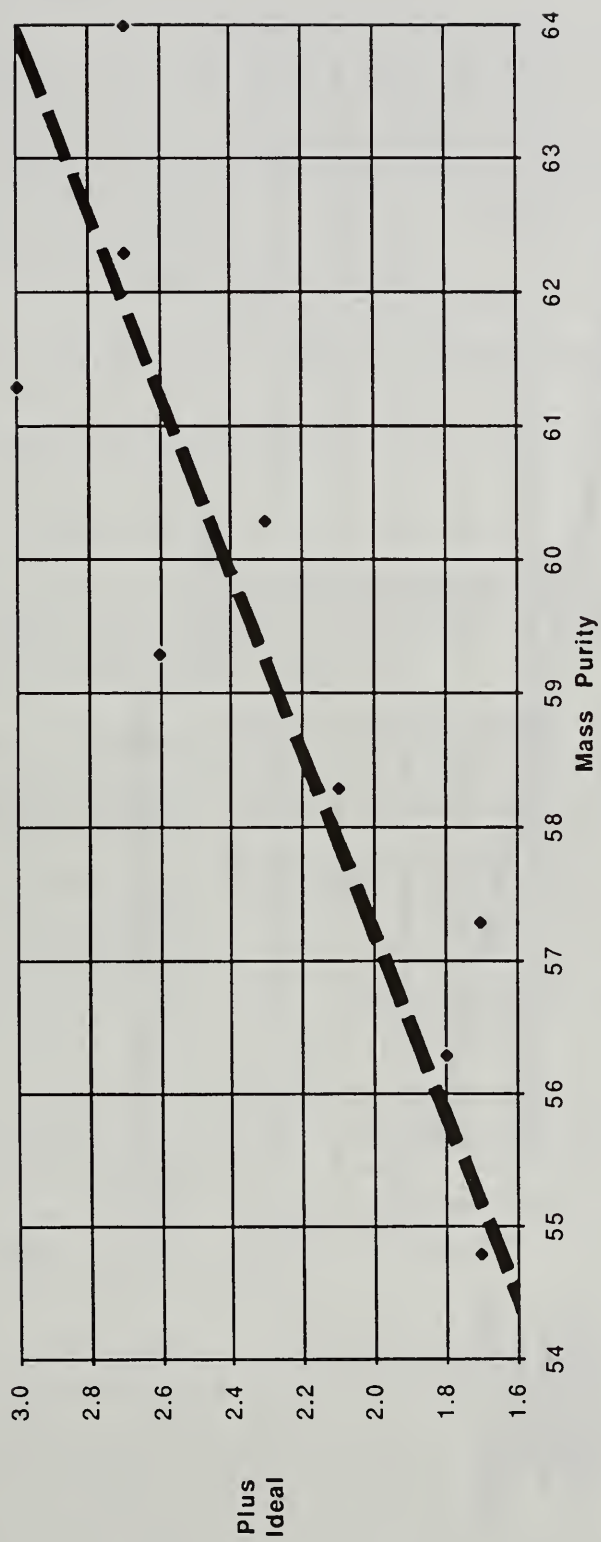
Graph IV

Graph Showing Effect of the Volume of Seed on
"Plus Ideal Difference"



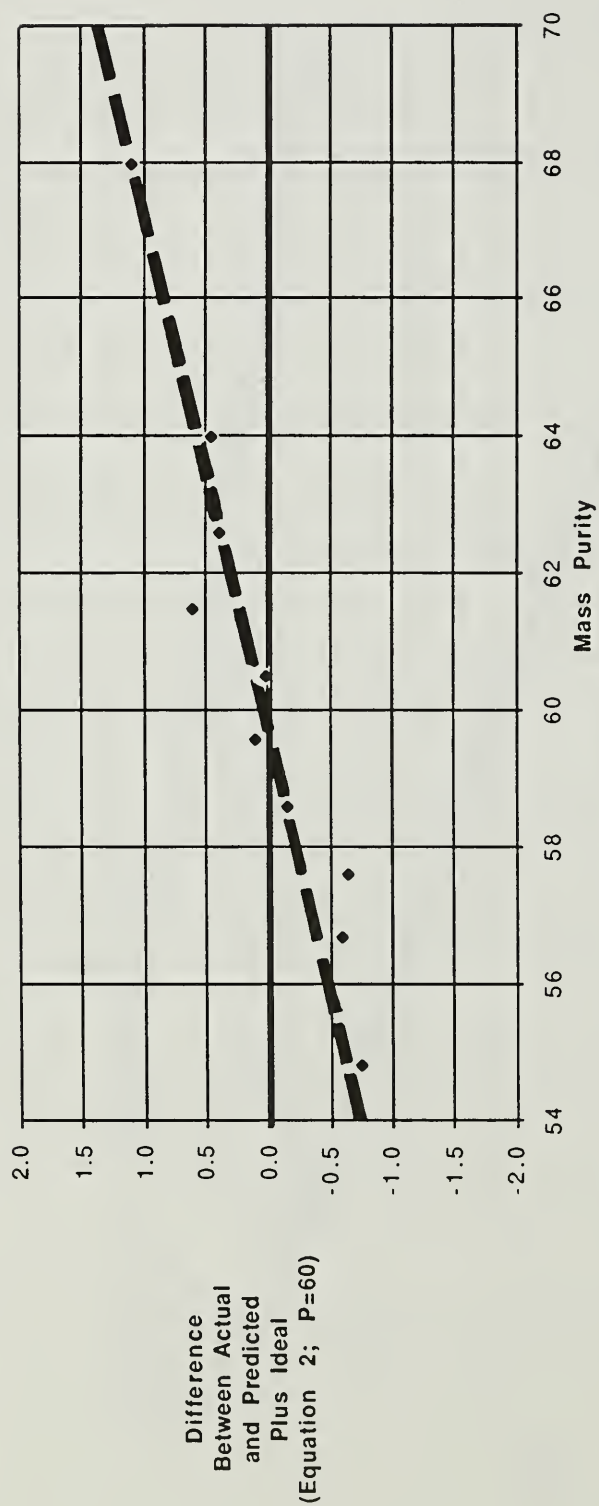
Note: Each point represents 20-60 strikes

Graph V
Graph Showing the Relationship of Plus Ideal
to Mass Purity



Note: Each point represents 20-60 strikes

Graph VI
Graph Showing the Effect of Mass Purity on
"Plus Ideal Difference"



Elongation, Intern. Sug. J. 5, 71, 1969). Since dextran inhibits sucrose crystallization, it follows that with high dextran content in low grade syrups, sucrose exhaustion would diminish. Thus a low axial ratio (viz.) b axis/c axis should also reflect a lessening of sucrose exhaustion.

In order to see the effect most clearly, the performance was expressed as the difference between actual and predicted performance (equation 2). Graph VII indicates that very low b/c ratios might have an adverse effect (positive difference). However, the multiple regression analysis showed this not to be statistically significant. The magnitude of any effect appears to be comparatively small.

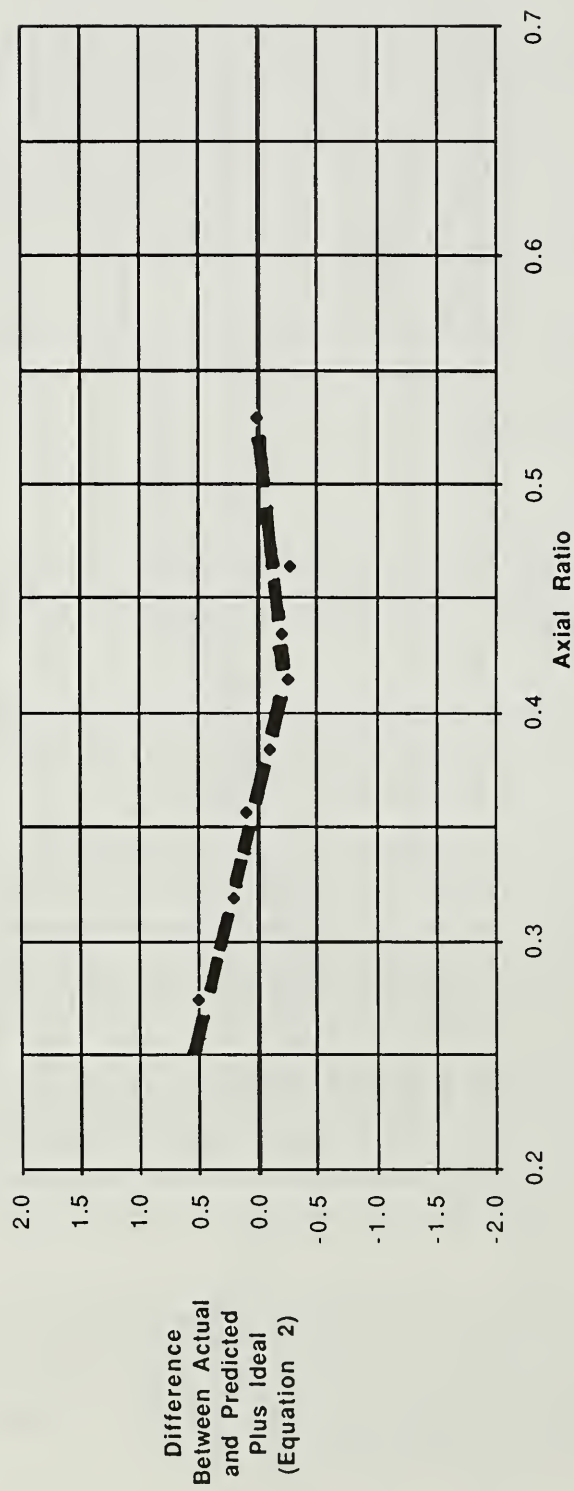
It may be noted here that at Refined Sugars the incoming raw sugar normally contains between 100-800 ppm dextran.

CONCLUSIONS FROM THE DATA

Several conclusions can be drawn from the data.

1. An increase of the invert/non-sugars ratio ("g") leads to a decreased sucrose exhaustion as measured by the Tate & Lyle "plus ideal" formula.
2. Within the parameters of massecuite purities used at Refined Sugars, the data show that when boiling at lower purities, better sucrose exhaustion was achieved.
3. In order to achieve good sucrose exhaustion (or lower the "plus ideal"), a certain minimum number of crystals per strike is required. We have found this minimum number to be in the area of 10×10^{11} . Higher crystal numbers did not appear to cause a significant improvement in sucrose exhaustion.
4. The individual crystal volume appeared to effect sucrose exhaustion since massecuites with lower average crystal volume had generally lower "plus ideal" values. This is a reflection of the same phenomenon as in the point above, i.e., larger crystal numbers mean smaller crystals with a larger total surface area.
5. Following our method of powdered sugar preparation, it was found necessary to use a minimum volume of 500 cc of seed sugar to effect good sucrose exhaustion. It has not been determined whether volumes of seed sugar in excess of 1000 cc will improve sucrose exhaustion.
6. Our data does not show a definite relationship between sucrose exhaustion and the axial ratio of the crystals. Further statistical analysis may show the effect more clearly.

Graph VII
Graph Showing the Effect of Axial Ratio on
"Plus Ideal Difference"



Note: Each point represents 20-60 strikes

OTHER APPLICATIONS FOR THE VIDS SYSTEM

Remelt

With respect to remelt strikes, in addition to the previously mentioned variables analyses, it is also possible to quickly determine the quality and consistency of grain strikes as well as the rate of crystal growth from grain strike to massecuite strike. It also became a simple matter to determine the distribution of crystal sizes and whether there was evidence of false grain, leading to a bi-modal distribution. Therefore, boiling procedures could be quickly modified to ensure maximum rate of growth.

Another use of the VIDS system could be to routinely check molasses samples from the centrifugal machines for the presence of crystals. If crystals are evident in the molasses from the machines, the screens can be checked for holes or other damage, corrections can be made, and so sucrose loss can be prevented.

Finally, strikes containing an excess of needle crystals can be correlated to the dextran content of the raw sugar in process.

White Sugar

At RSI we produce Extra Fine and Fruit Granulated Sugar for industrial use. The VIDS system can be used to measure crystal sizes of the white sugar strikes to monitor crystal distribution as done with remelt strikes.

Use of such data will allow us to vary volumes of powdered seed added to the strikes and to study the effect of seed volumes on growth rate, size distribution, and yield.

Effective control of crystal size distribution can prevent the development of fines which might have to be melted, leading to increased process costs.

Coated vs. Boiled Brown Sugar

Using the VIDS system, it is relatively easy to identify whether a brown sugar has been coated with syrup or boiled from runoff liquor since boiled brown sugars generally have a significantly smaller grain size than do coated browns.

Foreign Matter Identification

Since the VIDS television monitor provides a rather detailed clear picture of solid material on the slide, it can be very helpful in comparing foreign matter found in a product to standard slides of similar material and so can facilitate further chemical testing.

DISCUSSION

Nicholas Broughton, British Sugar plc - Speaking from the point of view of the beet sugar industry, I wonder if you could tell us a little more about the meaning of the "plus ideal" expression. I understand that it is some sort of measure of the exhaustibility. But I would like some amplification on that.

My second question relates to the influence of various parameters that you mentioned on this "plus ideal". At what point during the strike do you make these measurements? For example, when do you measure the crystal volume?

Dickey - We remove a sample when the pan is dropped. We put the material through a cyclone, and then read Brix, purity, etc. of the massecuite and the molasses, and from that calculate the yield or crystal volume.

The individual measurements on the VIDS system were done within about 24 hours of sampling. We initially did some laboratory studies to see if holding the samples caused changes, but we did not find any changes that were statistically significant. The crystal volume measurements have to be made immediately because once the massecuite cools, it can't be separated in a cyclone.

The "ideal" formula was developed empirically by Tate and Lyle (internal report, Plaistow Wharf, U.K.)

$$\text{Plus ideal} = \text{Total sugars} - \frac{100 (5 + 3g)}{3(3 + g)}$$

"g" is the ratio of invert to non-sugars. The basis was that sucrose exhaustion was essentially a function of g. We've found that g has the largest overall effect, but the mass purity also has an effect.

Enrique R. Arias, Sugar Cane Growers Cooperative of Florida - Can you tell me if your remelt recoveries improved during the course of, or since, this study?

Dickey - Yes. We've gained back about 1 unit ideal in total sugars.

Chung Chi Chou, Amstar Corporation - Congratulations on this fine work--it's really a big project. I have two questions.

First, what is the average total sugars (sucrose + invert) in blackstrap molasses on the 370 strikes? If this is confidential data, I will understand.

Joseph F. Dowling, Refined Sugars, Inc. - About 70%, dry basis, Clerget over solids by drying.

C. C. Chou - Secondly, what is the average crystal size on the final strike before you drop the pan?

Dickey - About 0.007 mm^2 . In the initial stages of this work, we saw an effect because of the small crystal number and large crystal volume.

RECENT LABORATORY STUDIES AT C & H SUGAR

Richard Riffer

California and Hawaiian Sugar Company

INTRODUCTION

This paper describes our recent laboratory studies in several areas, all of which share the common goals of improved refinery operations and better monitoring of such processes. Not every investigation led to an entirely successful conclusion, but we nevertheless felt there would be value in including such studies, because the purpose of these sessions is an exchange of information and ideas for the mutual benefit of participants. For the same reason, we have included some speculation for equivocal results in cases where several interpretations might be given.

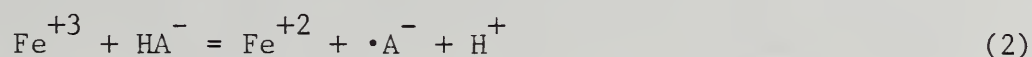
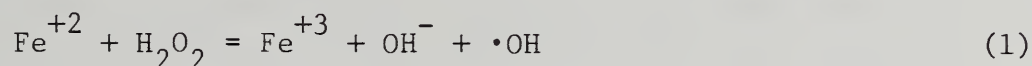
Recent areas of investigation include studies of iron catalysis in oxidative sugar loss, pan yield measurement using a tracer, and the use of chiral reagents in chromatography. Current dextran studies include improved sensitivity for an established technique and attempts at removal from sugar liquors. We have also developed an accurate method of carbon analysis for insoluble samples.

IRON CATALYSIS IN OXIDATIVE SUGAR LOSS

Introduction

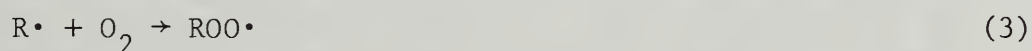
Iron is present in sugar liquors primarily as complexes with non-sugars, with intensely colored catechol adducts displaying a high degree of stability. Complexes with the hydroxy acids that can account for a large portion of the non-sugar fraction are by contrast very weak, readily disrupted by competing species such as phosphate. The fructose adduct is weaker still, and sucrose, in which all pairs of vicinal hydroxyls are trans, has little tendency to form an iron complex -- thus permitting the manufacture of granulated sugar with levels well under 1 ppm.

Our previous study of the consequences of iron contamination (Riffer 1984) demonstrated the effect of iron to be purely catalytic in autoxidative darkening. Only a small amount of iron is sufficient to propagate the branching of free radical chains, because reductive components in sugar liquors can regenerate Fe^{+2} :



However, very stable Fe^{+3} complexes such as phosphate would exhibit a reduced redox potential, thus inhibiting the Fe^{+2} regeneration process (Grinberg 1962, Neta and Simic 1985).

The highly reactive hydroxyl radical $\cdot\text{OH}$ can abstract H or add to double bonds at an exceedingly fast rate. We found that hydroquinone is a far better inhibitor than 1,4-benzoquinone, which suggested that the chains are propagated in part by peroxy:



In the studies reported here, we focused on invert, likely to be present in any low purity stream, since its components are far more subject to oxidative degradation than sucrose. Reactions were simulated using pure glucose or fructose, in buffered systems to eliminate pH effects of added ferric nitrate, a fairly strong acid -- the pH of a 1% solution being <2. Most experiments were performed at 70°C., which is on the low end of the range for refinery operations but permitted more careful monitoring of fast reactions.

Our initial studies were followed by polarimetry, but this was complicated by mutarotation and a multiplicity of reaction products; glucose does not -- as we had at first expected -- go primarily to gluconolactone. The reactions could also be followed by HPLC; we used an amino column with 80% CH_3CN as the solvent. For quantitative work it was essential to use an internal standard, preferably a non-reducing sugar that was a crystalline solid and displayed a convenient retention volume, cleanly separated from the peaks of interest. Erythritol satisfied these requirements, and although it was slowly degraded at 70°C., the rate was much lower than that for invert.

Iron in sugar liquors cannot be present as free Fe^{+3} because of the extremely small solubility product of ferric hydroxide. It must therefore be complexed with numerous and variable non-sucrose components, each with a characteristic redox potential that would influence the propagation of free radical chains. Because the nature of this population of complexes is not known -- although catechols probably contribute significantly -- laboratory simulations can at best give indications of the likely effects of iron contamination. We attempted to eliminate this

variable by carrying out parallel sets of experiments using different buffers of expected varying degree of interaction with iron.

Results

We found that in the pH range of 5-9, losses of sucrose and invert were not sufficiently accelerated by 100 ppm of iron to be detected using our HPLC technique. At pH 4, however, 9% of the glucose was lost in one hour at 70°C., compared to less than 1% in the absence of iron; fructose was not affected. About half the glucose loss could be accounted for by conversion to gluconolactone.

It was clear from our preliminary efforts that the catalytic effect of iron was more significant in color formation than in sugar loss. In pH 9 phosphate buffer, 50 ppm of iron increased the rate of color formation from fructose by 53% and from glucose by 30%. At 100 ppm, these figures were not increased much, probably because the solubility product of ferric phosphate had already been exceeded. (Color formation is reported here at the pH of the buffer used, not at the traditional pH 7. Thus results obtained at various pHs are not directly comparable.) We had expected the effects of iron to be self-limiting at elevated pHs as a result of its removal by insoluble salts of carboxylic acids formed by oxidation, but there was no evidence of this.

At pH 8, the effect of iron was more dramatic, perhaps reflecting its increased solubility. One might have instead predicted an effect smaller than at pH 9 because of presumed greater invert stability near neutrality. Iron at the 50 ppm level increased color formation from fructose by 148% whether the buffer was phosphate or MOPSO (3-[N-morpholino]-2-hydroxypropanesulfonic acid). With 100 ppm the rate of color increase in MOPSO was 218%. For glucose in MOPSO, the increase was 413% with 50 ppm of iron.

At pH 7 in phosphate buffer, 50 ppm of iron increased the rate of color formation in fructose by more than 140% but had no effect on glucose. When other buffers were used (MES (2-[N-morpholino] ethanesulfonic acid), PIPES (piperazine-N,N'-bis [2-ethanesulfonic acid), or MOPSO), the effect of iron was ambiguous because of high baseline ferric colors, absent in phosphate as a result of complexation.

Interestingly, addition of a stoichiometric amount of catechol to complex the iron strongly inhibited its effect on fructose in phosphate buffer, perhaps by two mechanisms -- reduced redox potential and disruption of free radical chain reactions. Under such conditions, iron increased the rate of color formation by only 40%, compared to 140% in the absence of catechol. With non-

phosphate buffers, the reaction mixture instantly turned inky from the iron-catechol complex.

The striking difference in the behavior of fructose and glucose might at first thought be attributed to the greater tendency of ketoses to form an enediol, but it must be remembered that this is not an alkaline solution. The difference might be due to the presence of cis vicinal hydroxyls in fructose, perhaps crucial to an oxidative intermediate. It is also possible that glucose forms only colorless products under these conditions.

At pH 6 in phosphate buffer, the effect of iron on fructose was smaller, a rate of darkening increased by 28% at 50 ppm. Again iron had no effect on glucose, and again data with non-phosphate buffers were difficult to interpret.

Finally, at pH 5 in phosphate buffer, color formation was very slow in both fructose and glucose, and 50 ppm of added iron was without effect. Since invert is degraded about ten times faster at pH 5 than at pH 6 at 70°C., it may be concluded that colorless products were being formed.

The results can be summarized as follows:

<u>pH</u>	<u>Effect of Iron</u>
4 - 5	Accelerated rate of oxidation of invert, especially glucose, with little effect on color
6 - 7	Sharply increased rate of color formation from fructose, but little effect on glucose
8	Strongly accelerated rate of color formation from invert, particularly from glucose
9	Increased rate of color formation from invert, but less dramatic than at pH 8

Under alkaline conditions fructose is degraded about 36 times faster than glucose (MacLaurin and Green 1969). The lower apparent sensitivity of fructose than glucose to iron suggests that heterolytic pathways are more likely to yield colorless products, and by rearrangement rather than by oxidation.

The fructose enediol can undergo β -elimination, with a high probability of forming polymeric conjugated enol structures (Fleming, Parker, and Williams 1971; Zideman, Bel-Ayche, Basch, and Lewin 1975; deWit 1976; and Bruijn, Kieboom, and van Bekkum 1986). Such systems would have an affinity for iron comparable to that of catechol and would strongly inhibit further color formation by homolytic processes.

RECIRCULATION OF IRON

Incoming raw sugar is only one of several iron sources in the refinery, and not necessarily the most important. Levels of 10^3 - 10^4 ppm are present in carbonaceous adsorbents and in magnesite. Iron oxides in surfaces contacting sugar, either in the solid state or in solution, are subject to dissolution or removal by abrasion. Low purity streams are particularly effective in this regard because of their relatively low pHs and relatively high levels of non-sugars containing ligands potentially able to coordinate iron. Recycled colorants, particularly those containing the catechol function, are likely to contain significant iron levels.

The kilning of carbon or bone char containing adsorbed iron-complexed colorant leaves an iron residue after the organic material is burnt off. Such buildups could contribute to micropore blockage. By what appears to be a parallel process, pyrolysis of adsorbed salts of carboxylic acids, high levels of calcium can be built up in granular carbon -- unexpected in an adsorbent that "doesn't de-ash."

We found that an acid wash of spent bone char, following the water wash but preceding kilning, removes only a small portion of this iron, the kinetics presumably being unfavorable. Alkaline washes, both sodium carbonate and sodium hydroxide, are even less effective. On this basis, one would not expect the iron to be leached out very efficiently by liquors during subsequent service.

If all colorants had equal polarity, mobility, and iron-coordination ability, iron removal would be proportional to color removal. However, adsorbents can be ranked for ability to remove complexed iron by measuring their ratios of iron removal to color removal using for example a standard soft sugar syrup. Although the result would be somewhat skewed if iron-containing color were inherently darker than iron-free colorant, such distortion is minimized by measurement of color at 420 nm, near the visible limit (Figure 1).

<u>Adsorbent</u>	<u>% iron / % color removed / removed</u>
styrene resin	0.487
granular carbon	0.409
Canesorb	0.368
acrylic resin	0.197

Granular carbon and Canesorb display similar behavior, as one would expect. Styrene resin, because of a higher level of aromatic structure, should exhibit a higher ratio than acrylic resin, and this is observed.

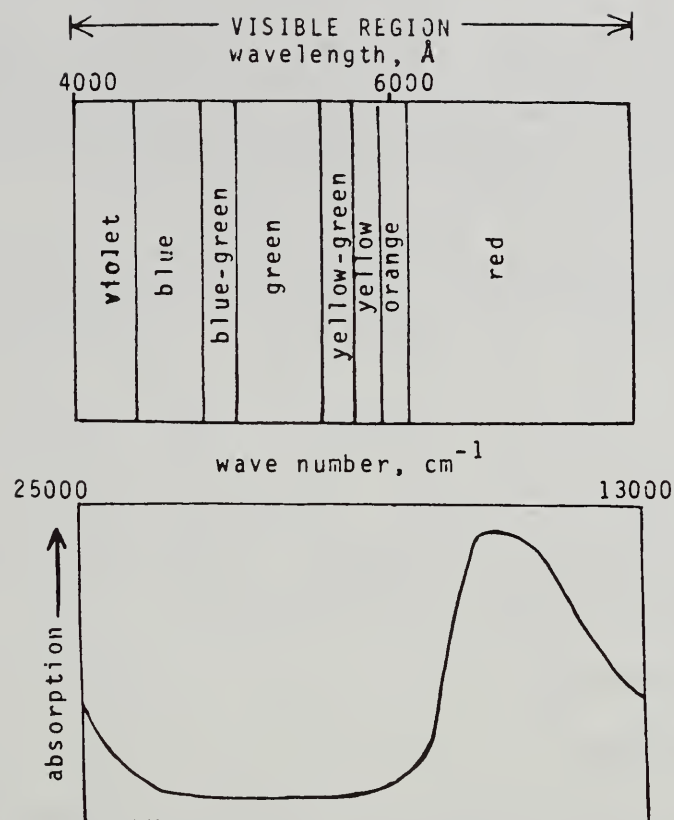


Figure 1.--The ferric-catechol complex absorbs light at each end of the visible region and transmits light in the middle, or green, region of the spectrum. Since neither ICUMSA 420 nor 560 measurements are near the maxima, "standard" color readings may not be particularly sensitive to iron-complex levels.

Kennedy and Smith (1976) found that styrene resin is better than bone char for removing derivatives of triclin but worse for those of luteolin. Since only the latter contain catechol groups, one would expect bone char to yield a higher ratio than the resin. However, when we tested new bone char, a small increase in iron level was observed in the test syrup, evidently from material leached from the char. Bone char seems to be unpredictable for iron removal, though it is not clear why a similar situation does not appear to arise with carbon, where perhaps the much higher surface area better favors a net removal. It is also possible that iron attachment on the colorant molecules could occupy sites that would otherwise serve as the locus for adsorption on the decolorizing medium.

The best strategy for iron removal would probably be the use of a combination of adsorbents, since none of them are highly selective. It is important to note that decolorizing resins have anion exchange capacity and remove phosphate and sulfate. This

should not only improve any subsequent decolorization on bone char (with any associated removal of iron), but should also reduce scale problems. However, on the other side of the ledger, a reduced phosphate concentration will also aggravate the consequences of remaining iron, if residual phosphate levels are insufficient to displace the iron from other non-sugar complexes. Greenish soft sugars could result.

In a related area, waste water streams can become reducing under anaerobic conditions. We have found that under such circumstances ferric iron complexes can be reduced to the less acidic ferrous forms. The latter commonly have larger dissociation constants, because donor groups tend to be basic. Dissociation is also favored by relatively low pHs. It is also possible that microorganisms utilize the organic portion of the complex, leaving the iron behind. If sulfates were initially present, such as from kilned proteinaceous material, ferrous sulfide ($K_{sp} = 10^{-19}$) can precipitate, together with the sulfides of any heavy metals present at trace levels.

POLYSACCHARIDES

Enzyme Studies

In the enzymatic control of dextran, endo-dextranases break the large dextran molecules into smaller fragments that have a reduced impact on pol, viscosity, and turbidity. Although it might be desirable to degrade the oligosaccharide fragments all the way to glucose, this is not ordinarily a practical goal. However, in dextran analytical procedures that make use of enzymes, the species ultimately measured is likely to be glucose. To degrade the initial products (oligosaccharides of D.P. 2-14) to glucose, α -glucosidase enzyme has commonly been used. This is not a particularly effective enzyme for the α -(1 \rightarrow 6) bonds of isomaltosaccharides, but it was probably the best choice among enzymes readily available commercially.

Within the past two years, isomaltase has become available from Sigma in St. Louis. This enzyme would be expected to degrade isomaltosaccharides much faster than does α -glucosidase. To test this, we assayed the two enzymes against isomaltose and followed the course of the hydrolysis using HPLC. To slow the reaction for ease of measurement, very low concentrations of enzyme were used in each experiment.

We found that glucose was produced 10.6 times faster with isomaltase than with α -glucosidase. When maltose rather than isomaltose was used as the substrate, α -glucosidase was 4.0 times faster. Thus isomaltase shows a greater specificity for α -(1 \rightarrow 6) linkages than does α -glucosidase for α -(1 \rightarrow 4) ones. Substitution of isomaltase for α -glucosidase would therefore increase the sensitivity of dextran procedures such as that of the Audubon Sugar Institute and the potentiometric method developed at C & H. α -Glucosidase would remain the enzyme of choice for

starch analyses in which glucose is the species ultimately measured.

The higher specificity of isomaltase might have been imposed by evolution. Hydrolytic rate data for maltose and isomaltose in dilute HCl (Pazur 1970) show similar activation energies, although maltose reacts about four times as fast at 80°C. Hydrolysis of the α -(1 \rightarrow 6) linkage could contain an intrinsically unfavorable entropy of activation term, perhaps because rotation about the C-5 to C-6 bond provides an extra degree of freedom absent in the α -(1 \rightarrow 4) linkage and subject to loss in the activated complex.

Characterization of Polysaccharides

Hawaiian raw polysaccharide levels appear to be fairly uniform, but qualitative differences, if present, could be significant. Specifically, the secondary linkages of highly branched components confer additional solubility on the molecules, which hinder removal in the refining process. The proportion of such molecules in waste streams would be expected to be greater than in the raws, and fluctuating levels could disrupt operations at our waste treatment facility. If bacterial DNA levels coding for carbohydrases are held constant and the enzymes are assumed to be of approximately equal molecular weight, then microorganisms capable of cleavage of several types of linkages could be less efficient than those of greater metabolic specificity.

To study degree of branching, we used concanavalin A immobilized on porous glass beads, using glutaraldehyde as a binding agent. Concanavalin A is a lectin that interacts only with highly branched polysaccharides. The immobilized preparation was found to remove most glycogen, a highly branched polysaccharide, from a mixture with a linear form, amylose.

When the procedure was used to study a set of raw cargoes, it was found that the polysaccharide fractions are fairly homogeneous and not highly branched. Thus they would not be expected to be responsible for variation in refinery or waste treatment operations. Polysaccharides responsible for high viscosities that inhibit oxygen transfer in waste treatment aeration basins appear to result from metabolic bottlenecks, such as nutrient imbalance, rather than from unusually refractory substances present in incoming raws.

Light-scattering Studies

Polysaccharide levels near the limit of detection using colorimetric or HPLC procedures were studied by direct turbidity measurement in the presence of alcohol. The alcohol haze technique is, of course, familiar as the basis of an analytical method for high molecular weight dextran.

Granulated samples very low in polysaccharides were spiked with known levels of high molecular weight dextran, pectin, or amylopectin. Molecular weights were demonstrated to be comparable by size exclusion chromatography. In each case the initial turbidity was low but, as expected, increased markedly upon addition of alcohol. However, the level of turbidity developed was much lower for pectin than for amylopectin or dextran. Furthermore, dextran and amylopectin displayed a 10-fold rise in dissymmetry upon alcohol addition, whereas no such increase was observed with pectin.

The dissymmetry is the ratio of scattering at 45° to that at 135° . Whereas readings at 45° and 90° are proportional to the concentration of the scattering species, the dissymmetry is relatively independent of concentration and is instead a function of the size and configuration of the scattering particles. Dissymmetry data must be interpreted with caution because for solutions (or sols) of macromolecules, the "scattering particles" may not be the molecules themselves but rather regions or domains of solute in which, owing to thermal motion, the concentration of solute is momentarily different from its bulk average.

To demonstrate that the increase in dissymmetry was not due to the refractive index change in going to a solvent containing alcohol, a measurement was made on an aqueous sample with Brix adjusted to the same refractive index as that of the alcohol sample. As expected, the high dissymmetry was not observed in this case.

The dissymmetry increase in alcohol suggested a large increase in the diameter of the scattering particles. This might at first thought be attributed to unfolding of linear molecules, which exist in solution as random coils, in analogy to the denaturation of a protein. However, most polysaccharide molecules are inherently "stiff" in solution. Furthermore, non-linear amylopectin cannot increase its molecular radius of gyration in a manner parallel to that of the more nearly linear dextran, and the sharp dissymmetry increase in alcohol indicated that the scattering particles were spherical, since such species display a much greater rate of increase in dissymmetry with maximum dimension than do other configurations.

The implication was that the larger particles were being formed by aggregation rather than by unfolding. The driving force would be the reduced surface area in the clusters to contact the less polar alcohol solution. The aggregates probably result from both specific intermolecular associations (hyperentanglements) and non-specific physical entanglements.

Pectin behaves differently from dextran and amylopectin, presumably because the molecules bear a high density of negative charge and hence repel one another. When other polysaccharides are present, pectin appears to impart a surface charge to the

aggregates, inhibiting cluster formation. We found that polygalacturonic acid, which has an extremely high charge-to-mass ratio, is a powerful inhibitor of dextran haze. It is possible that unusually high levels of indigenous sugarcane polysaccharide (ISP), which has a glucuronic acid content of about 10%, could result in low dextran results when the haze procedure is used. Suspect samples could be identified by zeta potential measurements.

Dextran Complexation

Dextran can be removed from solution by formation of insoluble adducts with heavy metal cations. Since both transition metals (copper) and main-group metals (lead) form such complexes, d orbital involvement is probably not essential; that is, metal \rightarrow ligand π bonds are probably not a requirement for such complexation.

In the copper aquo ion $[\text{Cu}(\text{H}_2\text{O})_6]^{2+}$, two water molecules are farther from the metal atom than the others; these bonds are very much longer and weaker than the other four and more readily exchanged with other ligands by successive displacement of water molecules. In the case of dextran, the resulting complexes are insoluble and precipitate. Alkaline conditions are required so that the dextran oxygen atoms are free to coordinate their electron doublets; in acid media, the oxygens are protonated and unavailable for complexation.

In the first transition series, the octahedral complexes are normally labile and come to equilibrium with additional ligands almost instantaneously. With such favorable kinetics, there seemed to be a chance that dextran could be adsorbed on copper immobilized on resin. However, without insolubility as the driving force, the affinity of copper for dextran hydroxyls would have to be much greater than that for water molecules. Unfortunately, this did not turn out to be the case.

There are analogous uses of weak coordination with cation resins to effect chromatographic separations. For example, resins in the calcium form are used to separate glucose from fructose, both commercially and in HPLC. Retardation of fructose with respect to glucose has been attributed to its relatively greater proportion of the open-chain structure in aqueous solution (Antikainen 1959, Verstraeten 1967). However, only very low levels of the open-chain forms exist, and we suspect the difference is due to the presence of *cis* hydroxyls in both pyranose and furanose fructose, but in only about 36% of the glucose, the α -pyranose form.

Paramagnetic copper complexes have recently been reported to have been used to stretch the tangled strands of polymers in an external magnetic field (Stupp 1986). If the linear backbones

of dextran molecules could be so aligned, it could dramatically reduce their solubility.

PAN YIELDS USING A TRACER

Pan yields can be estimated from a gross material balance, but the use of a tracer makes it unnecessary to know the weight of any of the fractions. The tracer fraction may be thought of as a dimensionless intensive property of the massecuite, sugar or syrup -- independent of the weight of total solids. If the pan yield is defined as the weight of sugar solids divided by the weight of massecuite solids, it can easily be shown that

$$\text{crystallization yield} = \frac{z - x}{z - y}, \quad (4)$$

where x, y, and z are the tracer fractions in the massecuite, sugar, and syrup, respectively. The equation is analogous to the familiar SJM formula for calculation of purities.

Note that the derivation does not require any assumptions to be made about the partition of tracer between crystals and syrup, such as its being identical to that of solids. This perhaps unexpected result is a consequence of the additional information provided by having three fractions. An exception to this would arise from the use of a radiotracer, such as carbon -14 enriched sucrose. This would obviously impose a requirement of distribution identical to that of unlabeled sucrose, because measurements of disintegrations per second per gram of sample, unlike parts per million, are not dimensionless.

The use of radiotracers in the refinery in any case would be a poor choice (not to mention one likely to be of enormous interest to the FDA). We instead chose as a tracer calcium, which can be measured at very low levels with high precision using atomic absorption spectroscopy (AA).

Because of the high sensitivity of AA, the number of significant figures in the result is limited by the refractometer reading used in determining the weight of the sample solids. For very high accuracy, the weight of centrifugal wash water used should be included in the yield calculation. The method obviously requires uniform distribution of calcium to avoid sampling error, but we did not find this to be a problem.

A VERSATILE SYSTEM FOR CARBON ANALYSIS

Introduction

For determination of Canesorb in mixtures with bone char, we have developed a modified Calgon procedure in which samples are separated into coarse and fine fractions using a 20-mesh screen, then subjected to hydraulic separation. Screening reduces the contri-

bution of particle size to the hydraulic properties of the granules and permits a sharp separation of Canesorb from bone char.

Carbon levels in bone char or other carbonaceous adsorbents are sometimes determined indirectly from weight loss after ignition in a muffle furnace at temperatures from 600-1000°C. We found this method to be not entirely satisfactory, even with very small, finely pulverized samples. The method is somewhat more reliable for bone char than for granular carbon, because the carbon in bone char is present as a thin layer on the hydroxyapatite and hence has a high exposed surface area. In any case, the weight loss method cannot distinguish between carbon and carbonate, which is only 20% carbon.

We further found that the presence of added pure hydroxyapatite sharply increases the loss of Canesorb carbon during ignition, presumably by interaction between calcium ions and Canesorb surface π electrons. This suggests that the presence of bone char increases Canesorb losses during kilning, particularly from fines. Losses could be minimized by avoiding unnecessary transport of dry adsorbent and the abrasion which that entails.

Commercial total carbon analyzers are designed for soluble organic carbon in aqueous samples, which makes them ideal for monitoring sugar losses. Carbonate may be determined indirectly by difference for samples that are not initially acidic. However, the method cannot be used for insoluble samples such as waxes, cellulosic material, or granular carbon.

We have designed a simple system suitable for the determination of total carbon and carbonate. The apparatus can also be employed in radiotracer work, for the preparation of samples for scintillation and planchet counting, and for ionization chamber measurements. Such methods are valuable in studying physical sugar loss, chemical degradation, and in determination of sucrose by isotope dilution.

Description of Apparatus (Figure 2)

The modified separatory funnel and reaction flask were constructed from commercially available micro glassware, such as Kontes (Vineland, NJ) Bantam-ware; glassware fabrication was done by Research and Development Products (Berkeley, CA). To insure freedom from leaks, the reaction flask and gas inlet tube are held firmly to the separatory funnel with yoke-type connectors, also available from Kontes. Ball and socket joints are secured with pinch clamps, and collection tubes with springs. Tygon tubing is used to join the flask side arm to the gas inlet tube. It is convenient to use a quick-disconnect joint near the midpoint of this tubing to facilitate dismantling.

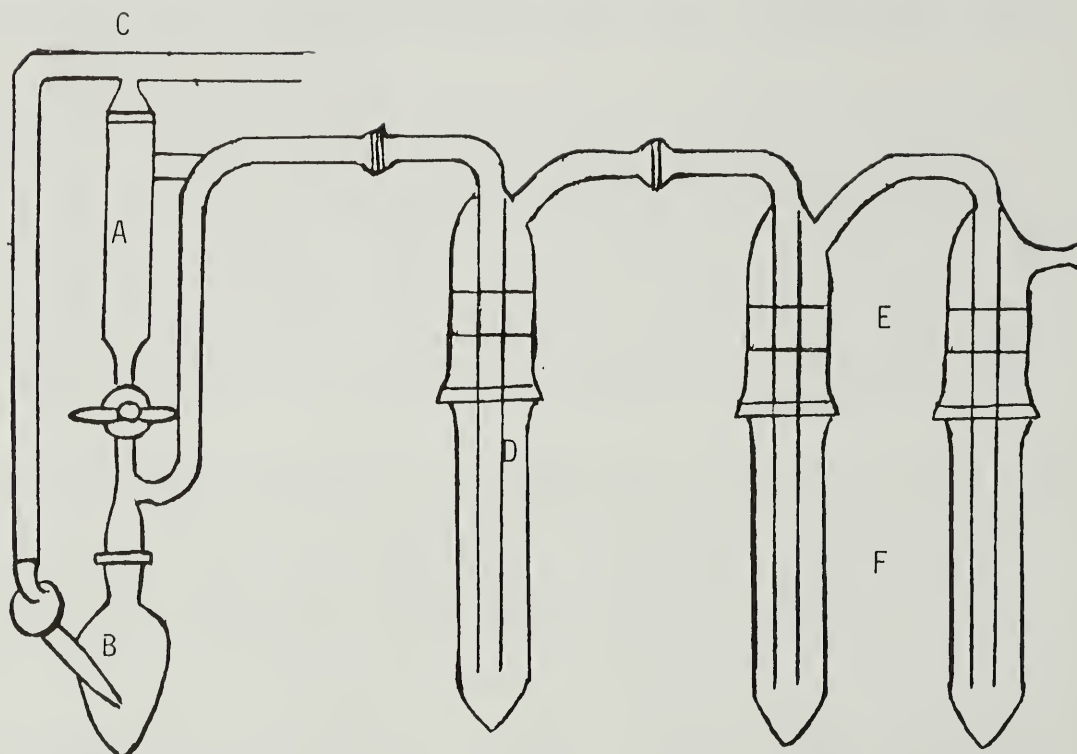


Figure 2.--Gas collection train. Gas generated when liquid from dropping funnel (A) reacts with sample in side-arm reaction flask (B) is swept through system by carrier gas introduced through tube (C). Washing tube (D) removes interfering substances, after which gases are collected in trap (E) containing two ground-joint centrifuge tubes (F). For collection of anhydrous gases, a U-drying tube may be inserted between D and E.

Determination of Total Carbon (See appendix)

Nitrogen, used as the carrier gas, is washed free of carbon dioxide in saturated barium hydroxide solution and dried over granular calcium sulfate. An Ascarite drying tube should be connected to the exit port of the gas collection assembly. The joint of the reaction flask can be lubricated with a drop of phosphoric acid, but organic lubricants must be avoided. A safety shield or visor is strongly recommended.

The sample is combusted by the van Slyke-Folch technique (van Slyke and Folch 1940), which makes use of a powerfully oxidizing mixture of hot sulfuric, phosphoric, iodic, and chromic acids. Most carbon containing materials are rapidly oxidized by this reagent; some exceptions are acetic acids, compounds that form acetic acid upon combustion, quaternary ammonium salts, and certain polymers such as polystyrene (van Slyke, Plazin, and Weisiger 1951; Dauben and Gee 1952). The carbon dioxide generated is trapped in sodium hydroxide, which is titrated with hydrochloric

acid after removal of carbonate by precipitation with barium chloride. This permits titration of a strong base rather than a weak one, since the bicarbonate endpoint is difficult to determine.

Nitrogen oxides generated from non-sugars can interfere with the result; in samples believed to contain substantial amounts of Amadori compounds, peptides, or amino acids, such oxides can be removed by a trap of precipitated manganese dioxide (Raaen, Ropp, and Raaen 1968).

Sulfur oxides do not appear to interfere: any sulfur trioxide that sublimes from the heated fuming sulfuric acid is readily decomposed by an aqueous trap. Little sulfur dioxide appears to form; although hot concentrated sulfuric acid is a strong oxidizing agent, any dioxide reduction product, despite its volatility, seemingly does not survive the highly oxidizing van Slyke - Folch environment.

THIN-LAYER CHROMATOGRAPHY STUDIES

Numerous reports of the separation of sugars by TLC have been published over the past 30 years. Although impressively long lists of separated carbohydrates are encountered in some of these articles, the separation of two of them of much interest to the sugar industry -- glucose and fructose -- is ordinarily not entirely satisfactory. A resolution that is only fair in quality may require elaborate plate preparation and multiple development. Although glucose and fructose are not diastereoisomers, the cyclic forms have identical numbers of hydroxyl groups and identical molecular weights, upon which their TLC mobility depends; hence resolution tends to be poor.

In the early years of TLC development, chemists commonly prepared their own plates using homemade spreading devices, but prepared plates have so come to dominate the field that commercial TLC-grade adsorbents are no longer readily available. Plates are rarely hand-coated today unless a special sorbent is required that cannot be obtained on commercial plates.

The separation of optical, geometrical, and structural isomers using bonded chiral* reagents is a rapidly expanding field (Yuasa et al. 1980, Wainer et al. 1980, Weinstein 1984, Grinberg and Weinstein 1984, Günter and Martens 1984, Armstrong 1985). At least one prepared chiral plate is already available (Ansper Co., Ann Arbor, MI), and another type is being planned (Armstrong 1986). We believed that the use of a chiral reagent that interacted with glucose and fructose offered the prospect of better resolution than has been possible until now.

*chiral = displaying right or left "handedness"

Using prepared "Chiralplates," we were able to achieve a good separation of certain amino acids, such as D- and L- α -amino butyric acids, but not of invert. The chiral selector in these plates turned out to be a proline derivative, which would restrict applications to amino acids, dipeptides, and certain derivatives.

β -Cyclodextrin (figure 3) is an attractive chiral selector because the ring of seven glucose units contains 35 chiral atoms, five per glucose unit. The toroidal structure has a diameter of 7.0Å, with 2-hydroxyl groups located at the entrance to the hydrophobic cavity. Because it is an oligosaccharide, some affinity for hexoses is not an unreasonable expectation. Bonded β -cyclodextrin has been reported to separate both enantiomers and diastereoisomers, but no reports have been published on sugars.

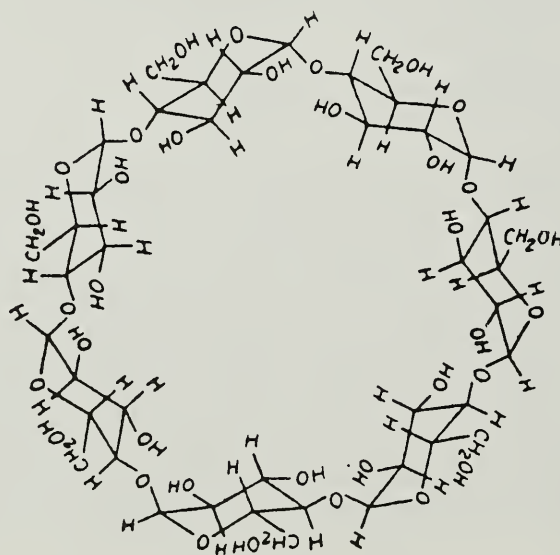


Figure 3.-- β -Cyclodextrin.

We attempted to achieve such separations using non-bonded material sprayed onto silica gel plates, the rationale being that covalently bonded plates would require elaborate preparation that would inhibit wide use. Furthermore, for non-preparative work there seemed to be no advantage in impregnating the adsorbent.

The plates were tested using various β -cyclodextrin levels and a number of solvent systems at various pHs. We were able to reduce the mobility of glucose and fructose by interaction with the chiral reagent, but not selectively. Neither were we able to resolve D- and L-glucose. A fairly high water content in the solvent system seemed to be important, perhaps affecting hydration of the toroidal cavity or the wetting angle. However, salts added to the solvent to facilitate hydrogen bridge formation between hydration envelopes of invert and sorbent were without effect. It is possible that cyclodextrin on silica gel preferentially assumes a disadvantageous orientation.

The fact that glucose and fructose did not display selective behavior suggested that a smaller cavity might be effective, so we examined α -cyclodextrin (six glucose units, diameter 5.5Å), also without success. To clarify the relationship between the sugar molecular size and the cavity diameter, we also studied separation of pentoses and of D- and L-glyceraldehyde, but felt we were getting too far afield from our original task. We plan to pursue this further.

A by-product of this research has been the discovery of a fairly good and simple conventional TLC separation of sucrose, fructose, and glucose: 85% acetonitrile on silica gel plates. Sharply defined and well-rounded spots are obtained. The chromogenic reagent is naphthoresorcinol.

Sugar	R _f value	R _f rel. to fructose	Color
xylose	0.43	1.54	blue
arabinose	0.36	1.29	blue
fructose	0.28	1.00	red
glucose	0.22	0.79	blue
sucrose	0.14	0.50	red

APPENDIX

Reagents for van Slyke-Folch Combustion

General reagent

Liquid: 67 ml. of fuming sulfuric acid (20% sulfur trioxide), 33 ml. of phosphoric acid, and 1 gm. of potassium iodate are heated to 160 - 190° until the salt has dissolved.

Solid: 2 parts of potassium iodate to 1 part of potassium dichromate.

Carbohydrate reagent

Liquid: 50 ml. of concentrated sulfuric acid, 50 ml. of concentrated phosphoric acid, and 1.5 gm. of potassium iodate are heated to 160 - 190° until the salt has dissolved.

Solid: 10 parts of potassium iodate to 1 part of potassium dichromate.

Combustion Procedure

Into the reaction flask are placed 100 mg. of sample and 1 gm. of van Slyke-Folch solid reagent; the sample size can be adjusted

according to the approximate carbon content. Five ml. of liquid reagent is pipetted into the separatory funnel, and nitrogen is passed through the system at 25 ml./min. The washing tube is filled with 1% stannous chloride in 5% sulfuric acid, to remove iodine from the effluent gases. The two gas collection tubes are each filled with 12.5 ml. of 0.200 N sodium hydroxide.

To begin the oxidation, the liquid is dropped into the reaction flask, and the mixture is heated gently for 90 seconds with a microburner flame. Some resistant samples may require as much as 10 min. of heating for complete combustion, but vigorous heating can be damaging to reproducibility.

Continue sweeping with nitrogen for an additional 20-30 min., then transfer the contents of the gas collection tubes carefully, with washing, to a 125 ml. flask. Add 5 ml. of 25% barium chloride and a few drops of phenolphthalein, and titrate with 0.100 N hydrochloric acid. A control blank should also be run.

Using an Ionization Chamber

A U-drying tube filled with anhydrous granular magnesium perchlorate (Dehydrite or Anhydrone) is placed in the train after the washing tube, and the gas collection assembly is replaced by a 250 ml. ionization chamber, using an appropriate adapter. Carbon dioxide is used as the carrier.

The chamber is evacuated and connected to the glass assembly. The pressure within the system is equalized by opening the chamber stopcock immediately before performing the combustion. Samples of moderate to high specific activity should be used, since in this case the volume of carrier gas is limited. After combustion, the system is returned to atmospheric pressure by opening the gas inlet to the carbon dioxide carrier.

Preparation of Samples for Scintillation Counting

The apparatus is the same as for carbon determination, but the collection tubes are filled with scintillator solvent containing 0.6N NCS solubilizer, a quaternary ammonium base in toluene (Amersham Corp., Arlington Heights, IL). The stannous chloride trap effectively removes iodine and other quenching substances. A magnesium perchlorate drying tube is recommended to keep from saturating the scintillator solution with condensed water vapor.

Preparation of Samples for Planchet Counting

The carbon determination procedure is used, but with tared gas collection tubes. After the carbon dioxide is collected, 2 ml. of 25% barium chloride is added to each tube. The tubes are centrifuged, and the barium carbonate washed thoroughly before drying and weighing.

It has been observed (Evans and Huston 1951, Zlotowski and Zielinski 1959) that isotope effects can accompany partial wet combustion, and in such cases the collected carbonate will not have a molar radioactivity that is representative of the sample.

Determination of Carbonate

The carbon determination procedure is used, but without van Slyke reagent. The stannous chloride wash tube is omitted, and 5 ml. of 50% phosphoric acid is used in the separatory funnel.

Decomposition of Labeled Barium Carbonate

The carbonate procedure can be used to convert labeled barium carbonate to carbon dioxide for collection in a scintillator solvent or in an ionization chamber. Nitrogen is used as the carrier gas, but non-labeled carbon dioxide should be substituted in the case of the ionization chamber.

Wet Combustion of Labeled Material

See also: Calvin et al. 1949, Collins and Ropp 1955, Jeffay 1962, and Wang and Willis 1965.

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DISCUSSION

A. P. G. Kieboom, University of Delft - With respect to the first part of your paper, I think there is a possibility that iron also catalyzes, by strong metal complex formation, the fructose splitting up into two C-3 moieties. That's a reaction we found for calcium (J. M. de Bruijn, A.P.G. Kieboom, and H. van Bekkum, Recl. Trav. Chim. Pays-Bas 105 (1986) 176-183).

Riffer - Yes, it may be. Of course, there are many reactions going on that don't result in color formation; I've dealt mostly with reactions that form color. Probably the reactions that don't result in color are more important in destruction of invert than those that do.

Andrew VanHook, College of the Holy Cross - Do metals other than iron that change valence readily also catalyze these color-forming reactions? I'm thinking of nickel and cobalt, which you would not expect to find in the refinery, but which might have the same chemistry.

Riffer - Yes, I'd expect those elements to have a similar activity, although as you say they would not be present in the refinery in significant concentrations. We have to remember that iron is more likely to catalyze oxidation than most elements other than copper.

RECENT STUDIES ON DEXTRANS AND POLYSACCHARIDES IN REFINERY PROCESSES

Margaret A. Clarke, Earl J. Roberts and Thanh B.T. To

Sugar Processing Research, Inc.

INTRODUCTION

The behavior of dextrans in sugar production and refining processes is of importance from the points of view of energy saving, processing efficiency and product quality.

This report includes observations in three areas of study: the effect of dextrans on polarization measurements in cane juice and raw sugars, the removal of dextrans of different molecular weight in various refinery processes and the recycling of dextrans in the refinery melt house. These studies are continuing.

EFFECT OF DEXTRANS IN POLARIZATION MEASUREMENTS

There has been some discussion in the literature about the effect of dextrans on polarization measurements. Dextrans are known to have a high specific rotation of approximately 200° at normal measurement temperatures, and therefore to increase a polarization measurement by about three times the value of an equivalent weight of sucrose. This has been clearly shown to be the case by Chou and Whukowski, where dextrans added to sugars (white and raw) were then separated by dialysis and tested for polarization. The experimental effect was found to be very close to the theoretical. This is also the case in polarization measurements on white sugars.

But to achieve this effect, the dextrans in the sugar must also be in the polarization tube. If the sugar sample is clarified before the measurement (which white sugars generally are not), then dextran may be removed during that clarification step. This has been observed by Guzman at Tucuman and Legendre in Louisiana, working on juices from deteriorated cane, where Herles' reagent (a basic lead nitrate mixture) was

used to clarify refractory juices. These juices, from stale or frozen cane, cannot be clarified well enough with lead acetate to allow a polarization reading to be taken. Guzman observed that the polarization of juice with and without added dextran or "cane gums" was the same after clarification with Herles' reagent--a surprising result, when increased polarization was expected from the dextran and gums. Legendre found that polarization results on juices from frozen cane clarified with Herles' reagent correlated very well with HPLC analysis for sucrose in these juices.

The reason for these observations is that the Herles' reagent carries the polysaccharides into the precipitate, whereby they are filtered off, and do not affect the polarization of the filtrate. The very basic reagent causes loss of sucrose and should not be used for regular polarizations.

Clarification with lead acetate presents a similar possibility: are the dextrans in juices and sugars removed in the lead acetate precipitate, or do they remain in lead and filtrate to affect the polarization reading?

Recent work by CSR Ltd. (Bradbury et al., 1986) has shown that dextran in raw sugars is removed to a great extent during lead clarification, and that the increase in polarization caused by what dextran remains in the leaded filtrate is generally less than one-third of the original. The polarization measurement would, therefore, be increased by less than one-third of the theoretical amount, i.e. an addition of 900 ppm dextran to a raw sugar caused a pol increase of 0.11 rather than the theoretical prediction of 0.27.

Initial results from studies at S.P.R.I. on removal of dextran from cane juice and raw sugars (Clarke et al., 1985, 1986) also indicate that lead acetate clarification (wet or dry lead procedure) removes some or all dextran from the sugar or juice sample.

Table 1.--Effect of lead acetate clarification on removal of dextran (T-2000) from cane juice. All dextran is as ppm on juice, Roberts' method.

Dextran added to juice	Total dextran in juice	Dextran after lead acetate clar.	% Dextran removed in lead clar.
Juice A. 0	27	3	88.9
500	558	6	98.9
1000	1169	0	100.0
1500	1649	16	99.0
Juice B. 0	53	4	92.5
500	622	23	96.3
1000	1142	15	98.7
1500	1701	4	99.8

Results shown in Table 1 indicate that in cane juice clarification almost all dextran is removed in the lead acetate precipitate, leaving only a few percent to affect juice pol. Dextran in juice will, therefore, affect the pol balance calculations for the factory very little. High dextran juices can, however, go on into the factory undetected, if no analysis other than pol and purity is done on the juice. Juices shown here are normal juices, with dextran added. Very poor juices, from stale or frozen cane, cannot be clarified with lead acetate, as mentioned above (Clarke, 1985, 1986).

In the case of raw sugar polarization, there appears to be less complete removal of dextran by lead acetate clarification than in the cane juice case. There are several possible reasons: the viscosity of juice is usually lower than that of the raw sugar pol test solution, and the relative amount of lead acetate per volume is higher. Filter aid is used in juice clarification, along with lead acetate. Suspended solids are much higher in juice, and so there is a greater volume of precipitate both per volume of sample and per weight of sucrose.

Table 2 shows data on a representative range of raw sugars of various polarizations, and the dextran in the sugars, as determined by the Roberts' Dextran Test (Roberts, 1983), before and after lead subacetate clarification, that is, the dextran levels in the whole raw sugar and in the lead filtrate. Results agree with those of Bradbury et al.

Table 2.—Removal of dextran from raw sugars by lead acetate clarification in pol reading.

Raw sugar pol	Dextran (ppm)	Dextran after lead acetate	% Dextran removed by lead clarification
97.27	366	58	84
98.49	450	190	58
98.64	241	47	80
98.68	1670	576	66
98.94	676	48	93

When known quantities of dextran of various molecular weights were added to raw sugars, and the mixtures clarified, the clarified filtrates were analyzed by both Roberts' method (Roberts, 1983) (for total dextran) and haze method (Amstar, 1984) (for high molecular weight dextrans). Results are shown in Table 3.

Table 3.--Effect of lead acetate clarification on removal of dextrans added to raw sugars.

Dextran added before clarifying		Dextran remaining after clarifying	
T-10	T-40	Roberts, ppm	Haze, m.a.u.
Sugar A	-- 241 ppm found--		
	in sugar	47	0
200	--	191	0
--	200	83	0
1000	---	147	0
--	1000	66	0
1500	--	446	0
--	1500	39	10
Sugar B	-- 676 ppm found--		
	in sugar	48	0
200	--	20	0
--	200	88	0
1000	--	576	20
--	1000	65	0
1500	--	477	0
--	1500	69	0

Very low molecular weight dextran (T-10) and medium low molecular weight (T-40) were used. Results indicate that the higher molecular weight dextrans are preferentially removed by lead subacetate, leaving lower molecular weight material in the lead filtrate to affect the pol reading.

This observation explains, in part, the variation noted in Table 2 on removal of dextran by lead acetate. There can be a wide range of molecular weights of dextran in raw sugar, and sugars with a lot of high molecular weight dextran may lose all that dextran in the lead acetate precipitate, while sugars with a greater proportion of lower molecular weight (and probably more soluble) dextrans may show only partial removal by the lead acetate clarification.

Dextrans formed in cane are generally of high molecular weight, and, although these can be broken down in process, dextrans in juice may be expected to have an average molecular weight higher than those in raw sugar.

Work is continuing in this area, in a study on removal of dextran by clarifying reagents that do not contain lead, since the days of lead usage are apparently limited. The effect of dextran in sugars on polarization measured by the high wavelength dark solution polarimeter is also under study.

DEXTRANS IN REFINERY PROCESSES

In 1981, Fowler described the flow of dextrans through a carbonatation refinery, considering the dextrans entering in raw sugar and those that might be produced in the plant, possibly in sweet waters. He found that carbonatation was the only refining unit operation to relieve significant dextran removal. He also found that dextran concentration built up in lower grade sugars and that very little dextran was formed in sweet water.

Fowler analyzed dextran content by the haze method (the best available at the time). His results therefore indicate the behavior of dextrans of molecular weight above approximately 20,000. Because the Roberts' test analyzes for total dextran, the difference in the readings of the two tests estimates dextrans of low molecular weight. It is important to define the calculation for the expression of haze dextran as ppm, because several different tests and calculations are available. The haze test for these studies uses the procedure outlined by Amstar Corp. in 1984; the result is expressed in milliabsorbance units equivalent to the m.a.u. read in a 5 cm cell. This m.a.u. is converted to ppm according to the formula,

$$\text{ppm} = \frac{\text{m.a.u.} + 118}{0.659}$$

based on a calibration curve of dextran with molecular weight 40,000 daltons (T-40). This calculation will give a high ppm result relative to calculations made using higher molecular weight standard dextran curves.

Results are herein reported of a survey made across two refineries, Refinery A, a phosphatation-bone char refinery, and Refinery B, a carbonatation bone-char refinery. Samples were obtained by routine methods, with no provision for timing flow across the various refinery steps, stored and transported on ice and analyzed at S.P.R.I. laboratories. All results are reported as ppm on Brix; no attempt was made at mass balance calculations. Samples were also analyzed for total polysaccharide (Roberts, 1980). Results therefore should be interpreted as trends overall, rather than as individual absolute cases.

Results from Refinery A are represented in Figures 1A, B and C. Complete results are tabulated in Appendix A. Roberts' dextran results are, as expected, rather higher than haze numbers. In some cases, haze results are higher than total dextran; it must be remembered that some ash components--and other alcohol insolubles--can interfere and cause a high haze reading. Ash components build up in low grade material and can be at levels higher than those removed by normal pre-treatment in the haze test. The other probable reason for this occasional observation is that samples represent different stages of melt.

Higher molecular weight dextran appears to remain a fairly constant level through process, as expected (Fowler, 1981), from raw sugar to fine liquor. The difference between the two readings shows that low molecular weight dextran is removed in affination, but not in phosphate clarification, adding to the non-sucrose brix load in clarified liquor. The decrease observed in the low molecular weight dextran levels across the evaporator station, both here and in the carbonatation refinery (Figures 2A, B and C), suggests that some of this material deposits with evaporator scale, or is removed on decolorization. That this decrease shows up in 2 out of 3 cases where light No. 1 liquor is evaporated to heavy offers support for the possibility that this very soluble dextran deposits with the evaporator scale.

In both refineries, buildup of all dextrans is observed through the pan house, from fine liquor to low grade syrups. The lower molecular weight dextran appears to behave in a manner similar to the higher, but results are not clear,

probably because of ash interference in the haze test.

In all cases, levels in fine liquor were quite close, by both tests, supporting the evidence that low molecular weight dextran remains, for the greater part, in syrup and continues through low grade boilings to remelt. The percent dextran removed, from raw sugar to fine liquor, is higher as measured by Roberts' test than by haze, showing that much of the dextran that is removed in the refinery is in the lower molecular weight range.

Results from the carbonatation refinery show much less regular trends than these from phosphatation. There is an indication that the low molecular weight dextran is removed in carbonatation and press filtration to a greater degree than the high molecular weight. Levels of dextran incoming in the raws on dates of sampling here were very low: 0 to 10 m.a.u., or 324 to 411 ppm by Roberts' test, so that absolute differences caused by sampling errors will show up as higher percentages of the dextran levels than they would were the incoming dextran levels higher.

RECYCLING IN THE MELT HOUSE

As part of an ongoing study on recycling of non-sugars by addition of melt sweet water and remelt sugar to the melter, melt house samples were collected from Refineries A and B and analyzed for dextran by the Roberts' method.

Sweet waters are known to be a potential source of dextran production, especially if held or stored below operating temperatures under conditions where Leuconostoc can grow. It was, therefore, to be expected that dextran levels might increase from washed raw sugar to melt liquor, through the addition of dextran in melt sweet water. However, in only two out of six sets of samples from Refinery A and in no sets from Refinery B was such an increase in dextran observed. In the overall refinery samples, given in Appendix A, in 50% of the cases no increase was observed. While timing of sampling can lead to erratic data, the trend here is clear in that both refineries appear to be doing a good job of maintaining sweet water in commendable condition, and practicing good hygiene so that dextran is not forming in these low Brix waters. Data are listed in Appendix B.

SUMMARY

This report includes observations data on three phases of studies on dextrans in sugar manufacture and refining. The effect of dextrans on polarization, when lead acetate clarification is employed, has been found to be almost negligible for cane juice, and well under 50% of the theoretical value for raw sugars. A survey of dextran across refineries has shown that low molecular weight dextran builds up in run-off syrups and may be part of evaporator scale. A survey of dextran recycled via refinery melters has shown that very little recycling occurs.

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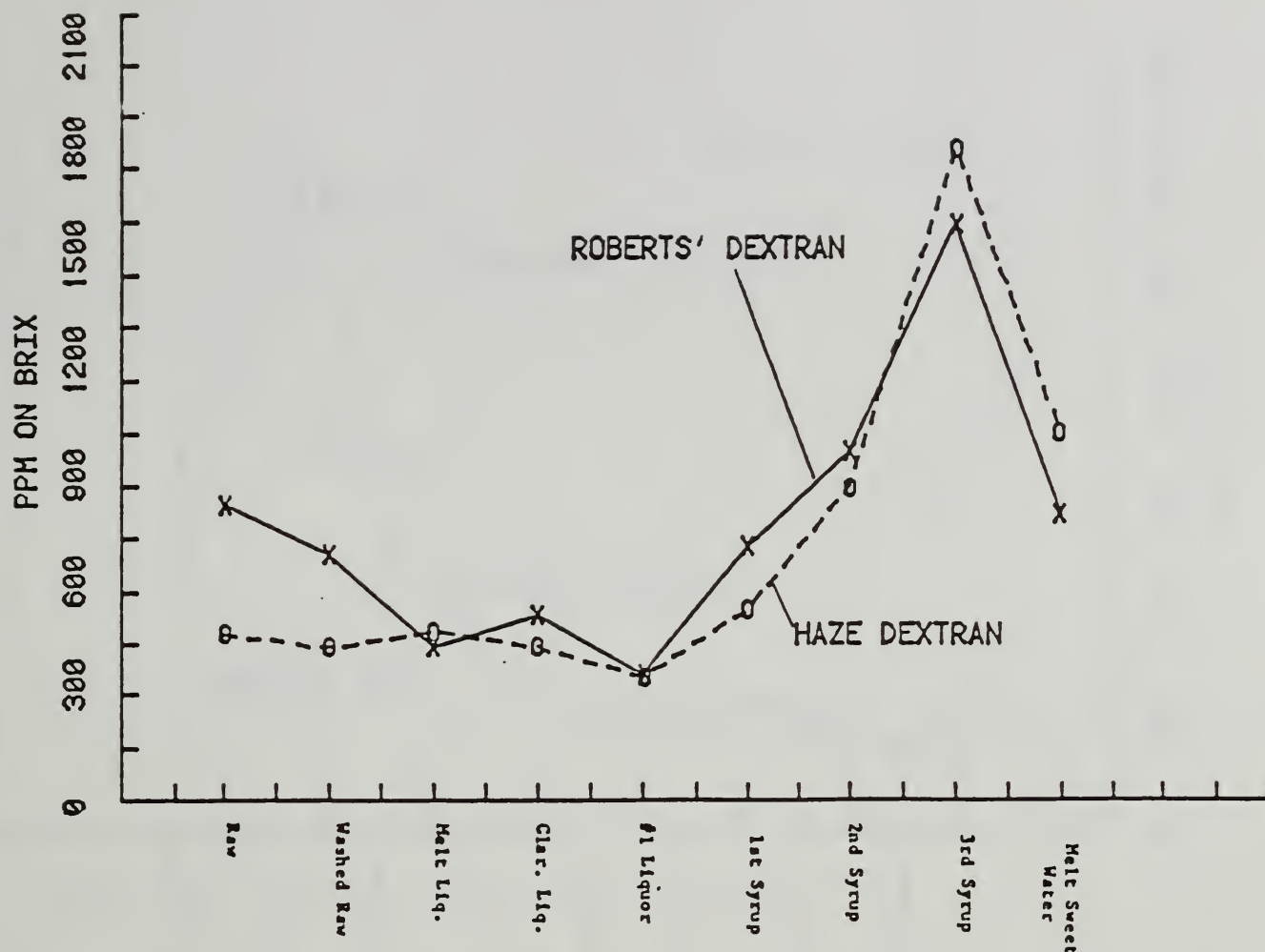


Figure 1A. Dextran in phosphatation refinery.

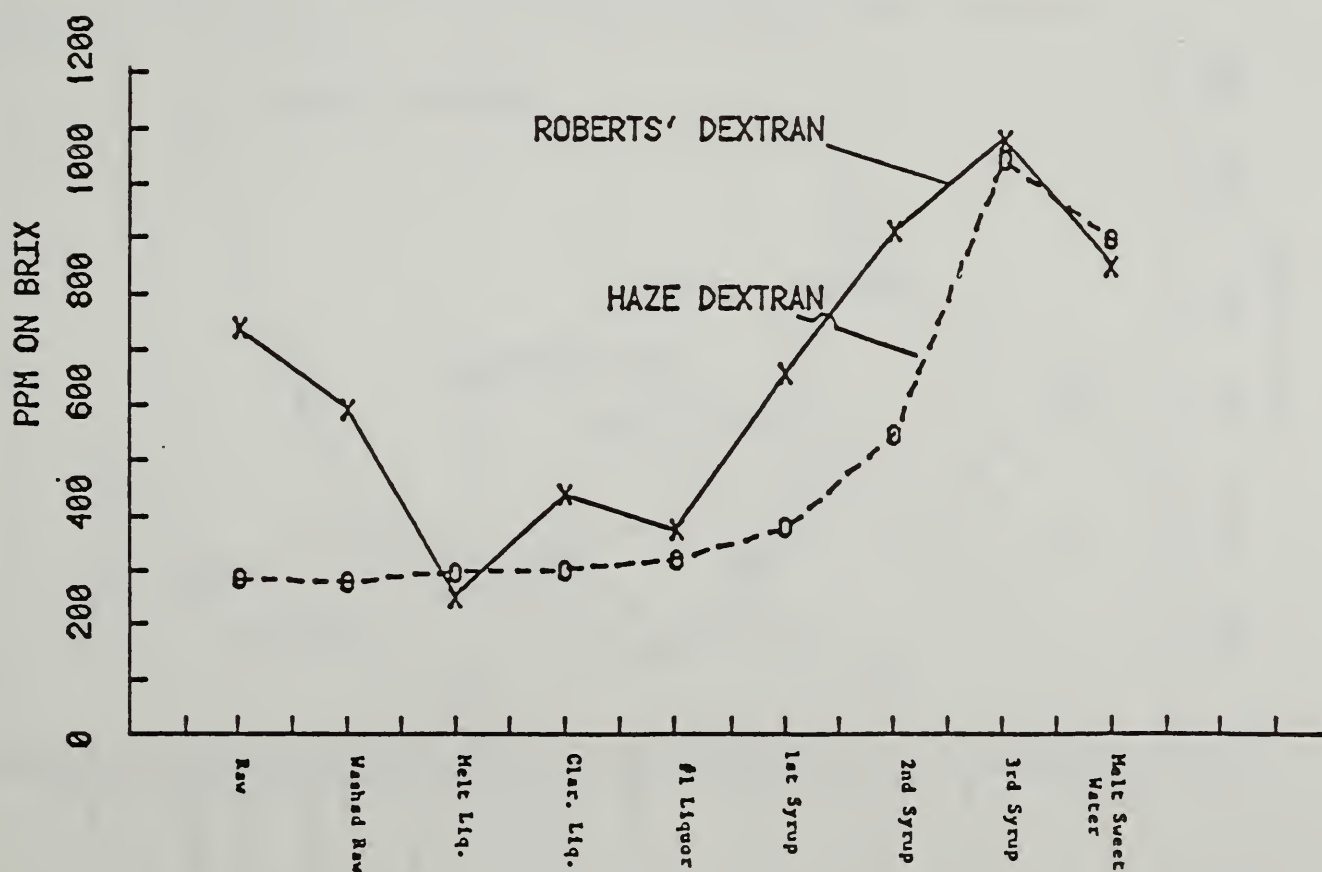


Figure 1B. Dextran in phosphatation refinery.

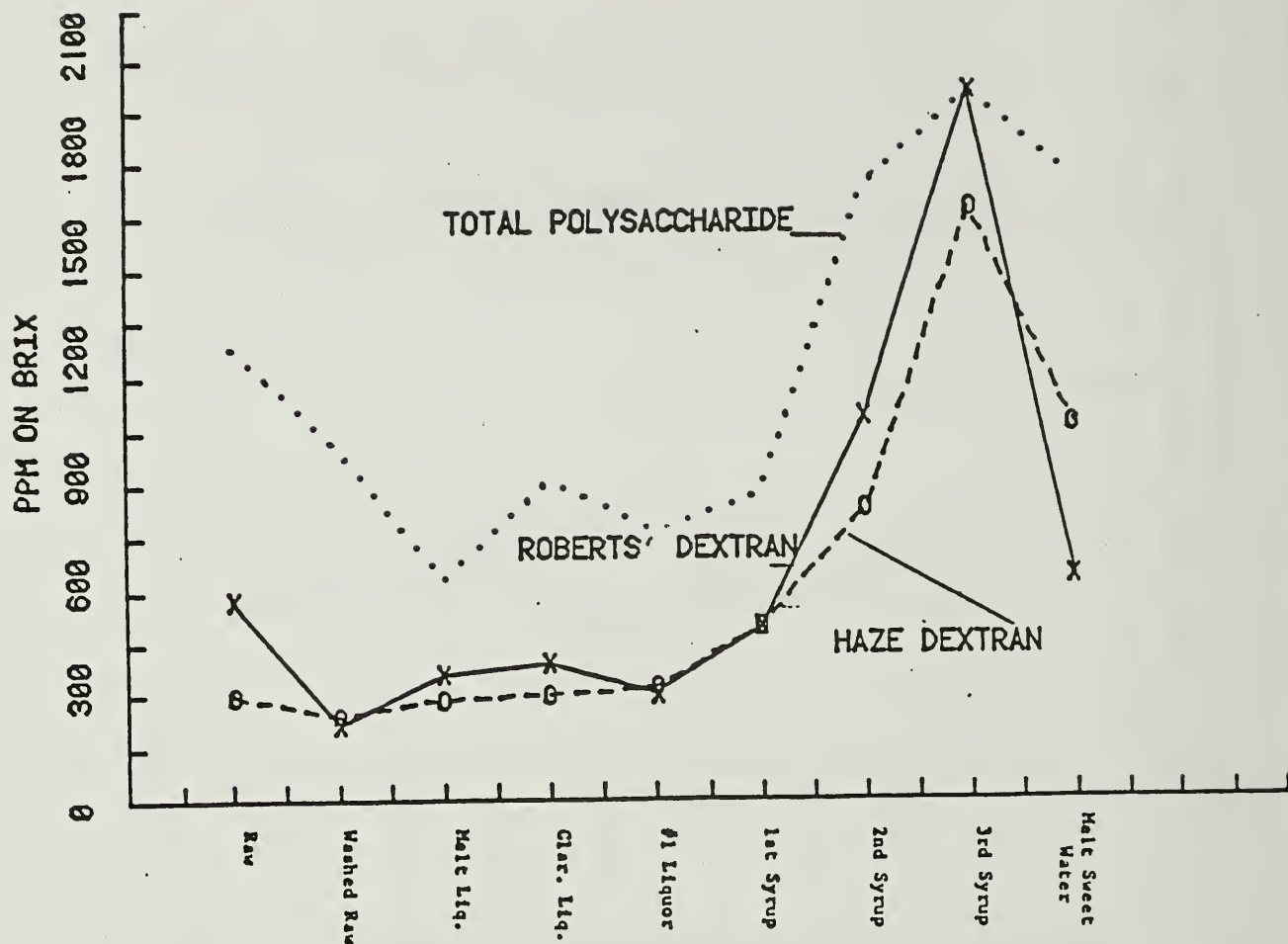


Figure 1C. Dextran and polysaccharides in phosphatation refinery.

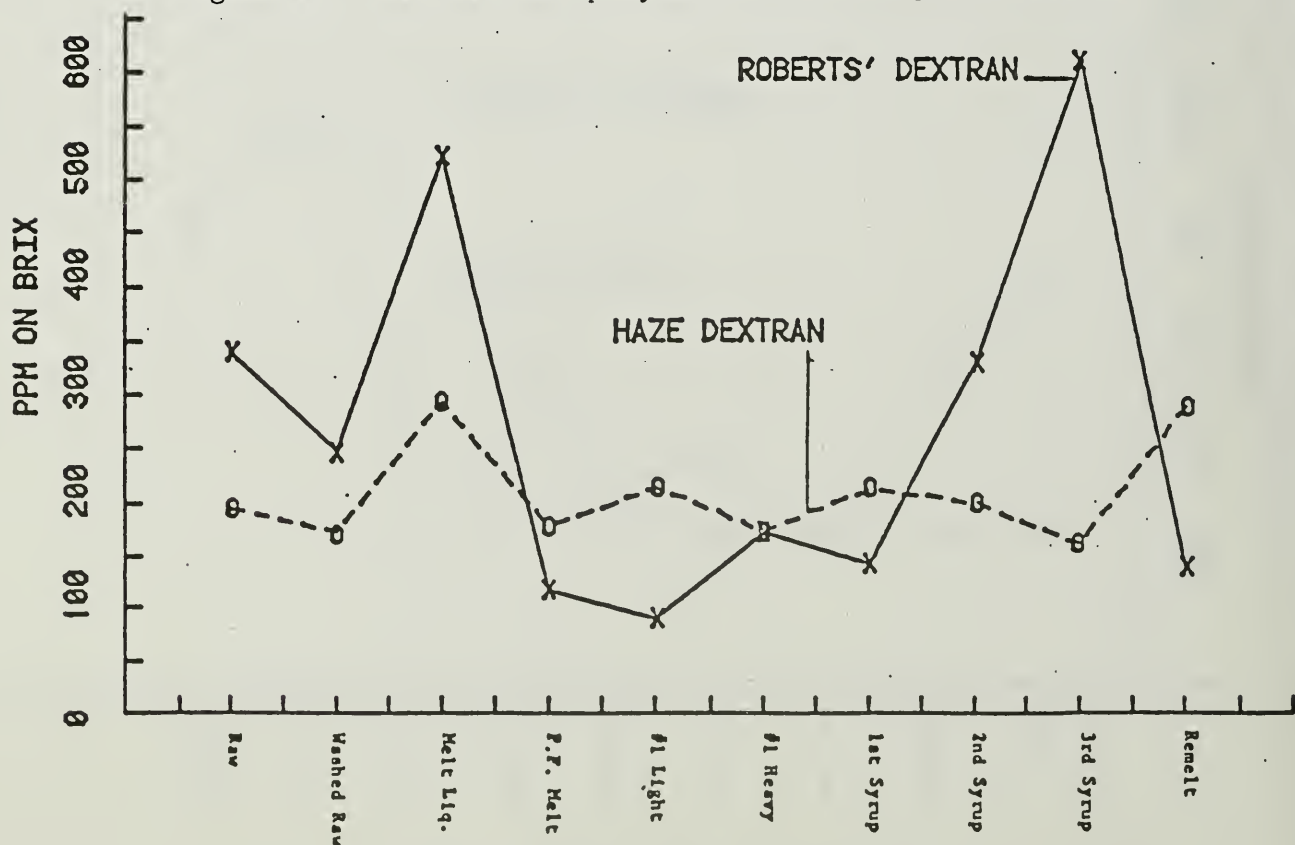


Figure 2A. Dextran in carbonatation refinery.

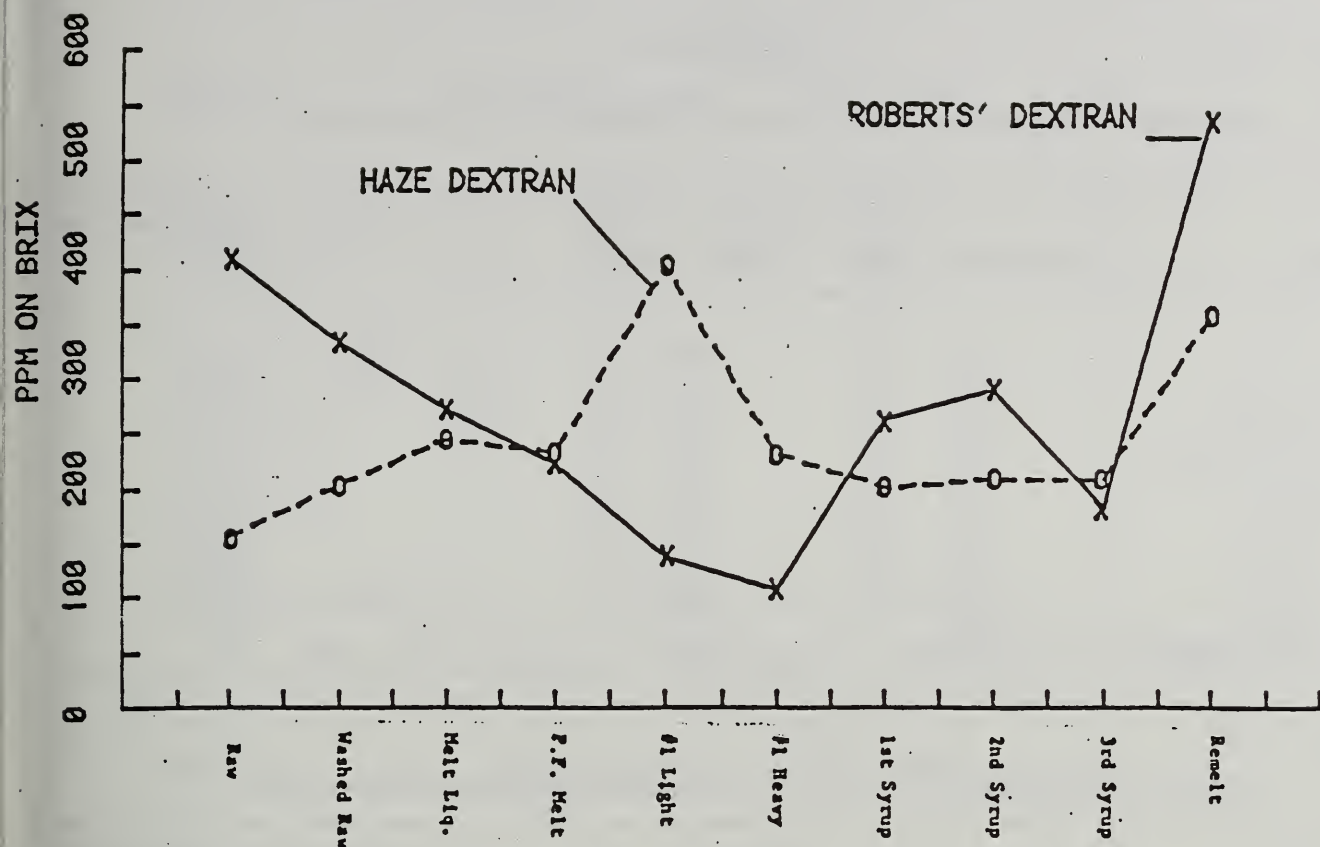


Figure 2B. Dextran in carbonatation refinery.

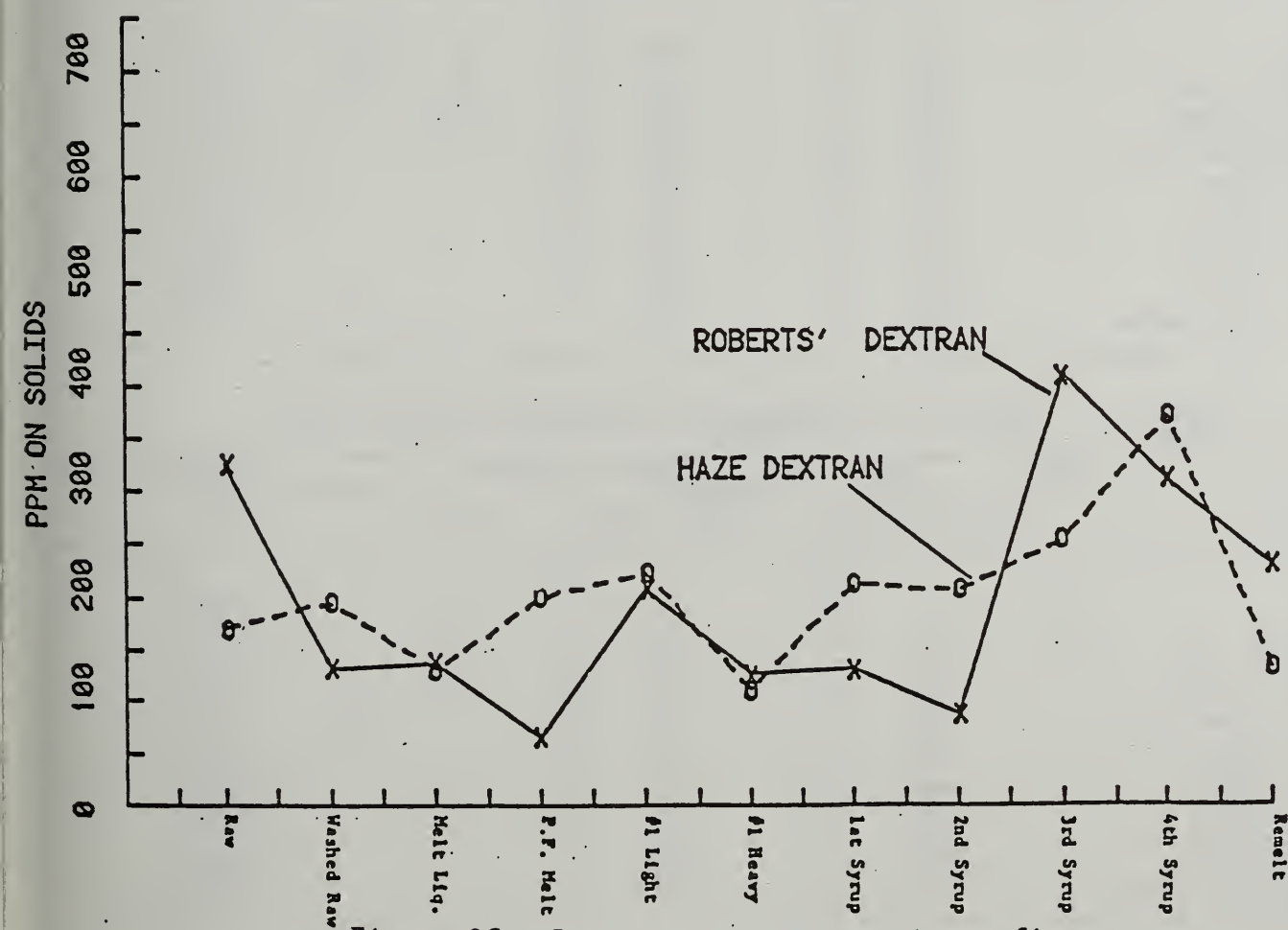


Figure 2C. Dextran in carbonatation refinery.

APPENDIX A

Table 1A.--Dextran in refinery A, phosphatation/bone char.

Sample	Roberts (ppm)	Dextran Haze (ppm)	mau	Total polysaccharide ppm
Raw	847	475	195	1933
Washed raw	704	437	170	2550
Melt liquor	438	484	N/A	909
Clarified liq.	530	436	"	1339
#1 liquor	357	350	"	1589
1st syrup	729	543	"	951
2nd syrup	997	898	"	1965
3rd syrup	1641	1858	"	4220
Melt sw. water	818	1051	"	1684

Table 1B.--Dextran in refinery A, phosphatation/bone char.

Sample	Roberts (ppm)	Dextran Haze (ppm)	mau	Total polysaccharide ppm
Raw	738	285	70	1174
Washed raw	595	278	65	1173
Melt liquor	252	297	N/A	1080
Clarified liq.	436	300	"	748
#1 liquor	372	318	"	512
1st syrup	658	376	"	962
2nd syrup	914	544	"	1862
3rd syrup	1079	1043	"	4146
Melt sw. water	852	898	"	1856

Table 1C. Dextran in refinery A, phosphatation/bone char.

Sample	Roberts (ppm)	Dextran Haze (ppm)	mau	Total polysaccharide ppm
Raw	573	300	80	1280
Washed raw	216	240	40	1007
Melt liquor	360	286	N/A	626
Clarified liq.	388	299	"	908
#1 liquor	300	318	"	764
1st syrup	492	492	"	874
2nd syrup	1086	826	"	1734
3rd syrup	2028	1666	"	2024
Melt sw. water	634	1062	"	1755

Table 2A.--Dextran in refinery B, carbonatation/bone char.

Sample	Roberts (ppm)	Dextran Haze (ppm)	mau	Total polysaccharide ppm
Raw	341	192	10	1102
Washed raw	247	171	0	586
Melt liq'r	522	293	N/A	1363
P.F. melt liq'r	117	177	"	310
#1 liq., light	90	213	"	574
#1 liq., heavy	172	175	"	630
1st syrup	142	212	"	666
2nd syrup	330	198	"	1434
3rd syrup	612	160	"	2735
4th syrup	1748	1187	"	5141
Remelt	139	290	"	643
Melt Sw. water	496	1843	"	394

Table 2B.--Dextran in refinery B, carbonatation/bone char.

Sample	Roberts (ppm)	Dextran Haze (ppm)	mau	Total polysaccharide ppm
Raw	411	156	0	2616
Washed raw	333	202	15	1619
Melt liq'r	271	243	N/A	684
P.F. melt liq'r	221	231	"	1890
#1 liq., light	136	203	"	1436
#1 liq., heavy	106	230	"	204
1st syrup	260	199	"	709
2nd syrup	289	205	"	2727
3rd syrup	1180	206	"	3849
4th syrup	1345	204	"	3676
Remelt	532	356	"	2197
Melt Sw. water	567	317	"	1865

Table 2C.--Dextran in refinery B, carbonatation/bone char.

Sample	Roberts (ppm)	Dextran Haze (ppm)	mau	Total polysaccharide ppm
Raw	324	171	0	2153
Washed raw	133	194	10	360
Melt liq'r	137	130	N/A	286
P.F. melt liq'r	66	198	"	209
#1 liq., light	206	222	"	242
#1 liq., heavy	126	111	"	271
1st syrup	130	211	"	261
2nd syrup	87	205	"	596
3rd syrup	409	253	"	1099
4th syrup	309	371	"	1809
Remelt	228	131	"	1633
Melt Sw. water	1102	243	"	1750

APPENDIX B

Table 1A.--Dextran levels in raw sugar melters.

Sample	Brix	Dextran, Roberts (ppm on Brix)
Raw sugar		686
Washed raw		682
Melt sweet water	17.0	1529
Melt liquor	69.2	316
Change, W.R.S. to melt liquor		-366
% dextran from recycled s.w.		

Table 1B.--Dextran levels in raw sugar melters.

Sample	Brix	Dextran, Roberts (ppm on Brix)
Raw sugar		1107
Washed raw		775
Melt sweet water	14.2	2667
Melt liquor	70.0	503
Change, W.R.S. to melt liquor		272
% dextran from recycled s.w.		

Table 1C.--Dextran levels in raw sugar melters.

Sample	Brix	Dextran, Roberts (ppm on Brix)
Raw sugar		448
Washed raw		304
Melt sweet water	24.1	884
Melt liquor	69.2	316
Change, W.R.S. to melt liquor		+8
% dextran from recycled s.w.		3%

Table 1D.--Dextran levels in raw sugar melters.

Sample	Brix	Dextran, Roberts (ppm on Brix)
Raw sugar		502
Washed raw		363
Melt sweet water	15.7	746
Melt liquor	63.9	271
Change, W.R.S. to melt liquor		-92
% dextran from recycled s.w.		

Table 1E.--Dextran levels in raw sugar melters.

Sample	Brix	Dextran, Roberts (ppm on Brix)
Raw sugar		641
Washed raw		521
Melt sweet water	19.1	695
Melt liquor	66.4	414
Change, W.R.S. to melt liquor		-107
% dextran from recycled s.w.		

Table 1F.--Dextran levels in raw sugar melters.

Sample	Brix	Dextran, Roberts (ppm on Brix)
Raw sugar		523
Washed raw		354
Melt sweet water	23.4	370
Melt liquor	66.5	1750
Change, W.R.S. to melt liquor		+1396
% dextran from recycled s.w.		

Table 2A.--Dextran levels in raw sugar melters.

Sample	Brix	Dextran, Roberts (ppm on Brix)
Raw sugar		521
Washed raw		511
Melt sweet water	6.4	348
Remelt sugar	49.1	1849
Melt liquor	67.0	296
Change, W.R.S. to melt liquor		-215

Table 2B.--Dextran levels in raw sugar melters.

Sample	Brix	Dextran, Roberts (ppm on Brix)
Raw sugar		1103
Washed raw		854
Melt sweet water	5.7	303
Remelt sugar	68.7	789
Melt liquor	66.1	478
Change, W.R.S. to melt liquor		-376

Table 2C.--Dextran levels in raw sugar melters.

Sample	Brix	Dextran, Roberts (ppm on Brix)
Raw sugar		938
Washed raw		725
Melt sweet water	2.1	262
Remelt sugar	66.1	912
Melt liquor	65.3	461
Change, W.R.S. to melt liquor		-266

Table 2D.--Dextran levels in raw sugar melters.

Sample	Brix	Dextran, Roberts (ppm on Brix)
Raw sugar		880
Washed raw		738
Melt sweet water	4.4	106
Remelt sugar	62.4	552
Melt liquor	65.8	339
Change, W.R.S. to melt liquor		-399

DISCUSSION

Enrique R. Arias, Sugar Cane Growers Cooperative of Florida - Thank you for continuing to work on dextran--we are also very interested in dextran as you know. Can the dextran molecule be broken down in the refining process or in the raw sugar house? We talk about removing dextran, but are we physically removing it or are we breaking it down or destroying it somewhere?

My second question: When we deal with high ash-low purity materials, how reliable are the haze and the Roberts' methods?

Clarke - To answer the second question first. In low purity materials where there are high ash levels--particularly high calcium sulfate levels--the haze analysis can be affected. The haze method includes a step using ion exchange resin to remove ash, but in very low purity materials, the ash level can be too high; all ash will not be removed, and the haze reading will be artificially high. The Roberts' test is not affected by high ash.

On your first question, I assume you mean possibilities of dextran removal other than a dextranase enzyme treatment. There is no record of naturally occurring dextranase in cane; there is natural amylase of course. Since dextran is not a product of metabolism of the cane plant, but comes from an outside source, there is no reason to expect a dextranase enzyme to occur naturally in the plant. I expect dextran can be broken down by heat--like starch--through thermal degradation, particularly in process areas where there are a lot of heating coils and not much circulation.

Mark Wnukowski, Amstar Corp. - A few years ago, we looked at blackstrap molasses, and the effect of lead acetate on removal of dextran. In some cases, dextran was removed and in other cases not. We used the haze method of analysis. Do you think the ash levels were interfering?

Clarke - You really don't know what the haze results mean in low purity materials like blackstrap. In high purity materials you can use the haze test more satisfactorily, but with blackstrap you can't tell when you have ash interference. One possibility is to change the amount of ion exchange resin in the haze test. If you increase the amount of resin, and the haze result decreases, then the initial result reads high because of alcohol-insoluble ash.

Whukowski - Could the evaporator loss of dextran be simulated using a rotary evaporator? What if a scale inhibitor were added? Could you then determine if the dextran went into the scale?

Clarke - These are good topics for research projects.

MECHANISM OF THE ALKALINE DEGRADATION OF MONOSACCHARIDES

J. M. de Bruijn, A. P. G. Kieboom, and H. van Bekkum

Delft University of Technology

INTRODUCTION

It has been known for almost a century (Lobry de Bruyn and Alberda van Ekenstein 1895, Nef 1907, 1910) that monosaccharides are unstable in alkaline medium and undergo, besides isomerization, degradation reactions which irreversibly lead to carboxylic acid products. The mechanism of these degradation reactions, however, has been only partly elucidated up to now (De Bruijn et al. 1986) because a complete and quantitative analysis of the reaction mixture was not possible. Recently, a routine HPLC analysis method (De Bruijn et al. 1984) was developed which, in some cases combined with GC analysis, allows a quantitative determination of the carboxylic acid composition of the final alkaline degradation mixtures of monosaccharides. In this way, a systematic investigation on the influence of several reaction parameters on the degradation pattern was performed (De Bruijn et al. 1986) from which the following features became apparent.

- (i) Formation of oligomeric acidic products, the so-called $> C_6$ acids, takes place at the cost of the well-known C_1 to C_6 acidic products (like lactic acid and saccharinic acids) and is maximal (up to 50%) when the HO^- concentration lies between 10^{-3} M and 10^{-2} M.
- (ii) Initial monosaccharide concentrations lower than 10^{-2} M result in an increased formation of saccharinic acids and lactic acid at the cost of the $> C_6$ acids. This implies that the precursors of saccharinic acids and lactic acid, i.e. α -dicarbonyl intermediates, are also involved in the aldolization reactions leading to the $> C_6$ acidic products.
- (iii) An HO^- concentration higher than 10^{-1} M results in a sharp increase in lactic acid production together with a somewhat higher formation of saccharinic acids. This at the cost of the $> C_6$ acids and of acetic acid, glycolic acid, and formic acid. In strongly alkaline medium the aldolization reaction is of minor importance and the α -dicarbonyl intermediates almost completely undergo benzilic acid rearrangement. In addition, the increased

amount of lactic acid is partly due to an enhanced direct retro-aldolization of ketoses.

- (iv) Coordination of monosaccharides by divalent cations, as proven for calcium in the case of D-fructose, may favour the retro-aldolization reaction. Apart from the resulting enhanced production of lactic acid, the interaction of calcium(II) with α -dicarbonyls affects the benzilic acid rearrangement/dicarbonyl cleavage ratio of these intermediates as demonstrated by the formation of branched saccharinic acids at the cost of acetic acid and glycolic acid.
- (v) Alkaline degradation experiments with pyruvaldehyde, glyceraldehyde, and 1,3-dihydroxyacetone as the starting compounds, all assumed to be reaction intermediates, indicate that aldolization of (di)carbonyl compounds causes the formation of substantial amounts of $> C_6$ acids.
- (vi) The nature and structure of these $> C_6$ acids, having average molecular weights of ~ 350 , ~ 500 , and ≥ 700 , further point to formation via aldolization of (di)carbonyls like pyruvaldehyde, 3-deoxyhexos-2-ulose, formaldehyde, acetaldehyde, glycolaldehyde, and monosaccharides.
- (vii) The kinetics of the alkaline isomerization and degradation of monosaccharides show that the alkaline isomerization/degradation of D-glucose, D-mannose, and D-fructose also includes the isomerization to and the degradation of D-psicose. Irrespective of the starting monosaccharide, substantial amounts of acidic products, i.e. $\sim 65\%$ and $\sim 20\%$, are formed via D-fructose and D-psicose, respectively.

On the basis of the foregoing new insights in the alkaline degradation of monosaccharides this paper will discuss the mechanism of the various degradation reactions, the effect of reaction parameters on the degradation pattern, and the role of α -dicarbonyl compounds as key-intermediates. Additional information from ^{13}C NMR spectroscopic (De Bruijn 1986) measurements of alkaline degraded 1- ^{13}C -D-glucose will be presented and discussed.

MATERIALS AND METHODS

1- ^{13}C -D-glucose, containing 99% ^{13}C , was obtained from C.E.A., France. Under N_2 at 78 °C and at constant HO^- concentration 340 mg 1- ^{13}C -D-glucose (0.025 M) was degraded in 75 ml aqueous 0.01 M KOH as described elsewhere (De Bruijn et al. 1986). After complete conversion of the monosaccharide (7 h) the neutralized reaction mixture was freeze-dried. ^{13}C NMR spectra of this reaction mixture in D_2O were recorded on a Nicolet NT-200 WB spectrometer (50 MHz) using dioxane as external standard ($\delta = 66.6$). Assignment of the signals was performed by comparison of the chemical shifts with those of authentic compounds (De Bruijn 1986). Quantitative ^{13}C spectra were

obtained by using gated ^1H -decoupling, a pulse width of 12.0 μs (45° flip angle), and a pulse delay of 100.0 s.

RESULTS AND DISCUSSION

Pattern of ^{13}C Label upon Alkaline Degradation of $1\text{-}^{13}\text{C}$ -D-Glucose

The ^{13}C NMR spectrum of alkaline degraded $1\text{-}^{13}\text{C}$ -D-glucose (De Bruijn 1986) has given the distribution of ^{13}C , originating from this labeled compound, over the different functional groups in each of the $\text{C}_1\text{-C}_6$ acids (Table 1). This ^{13}C distribution in the $\leq \text{C}_6$ acids offers further insight into their way of formation from D-glucose (Figure 1).

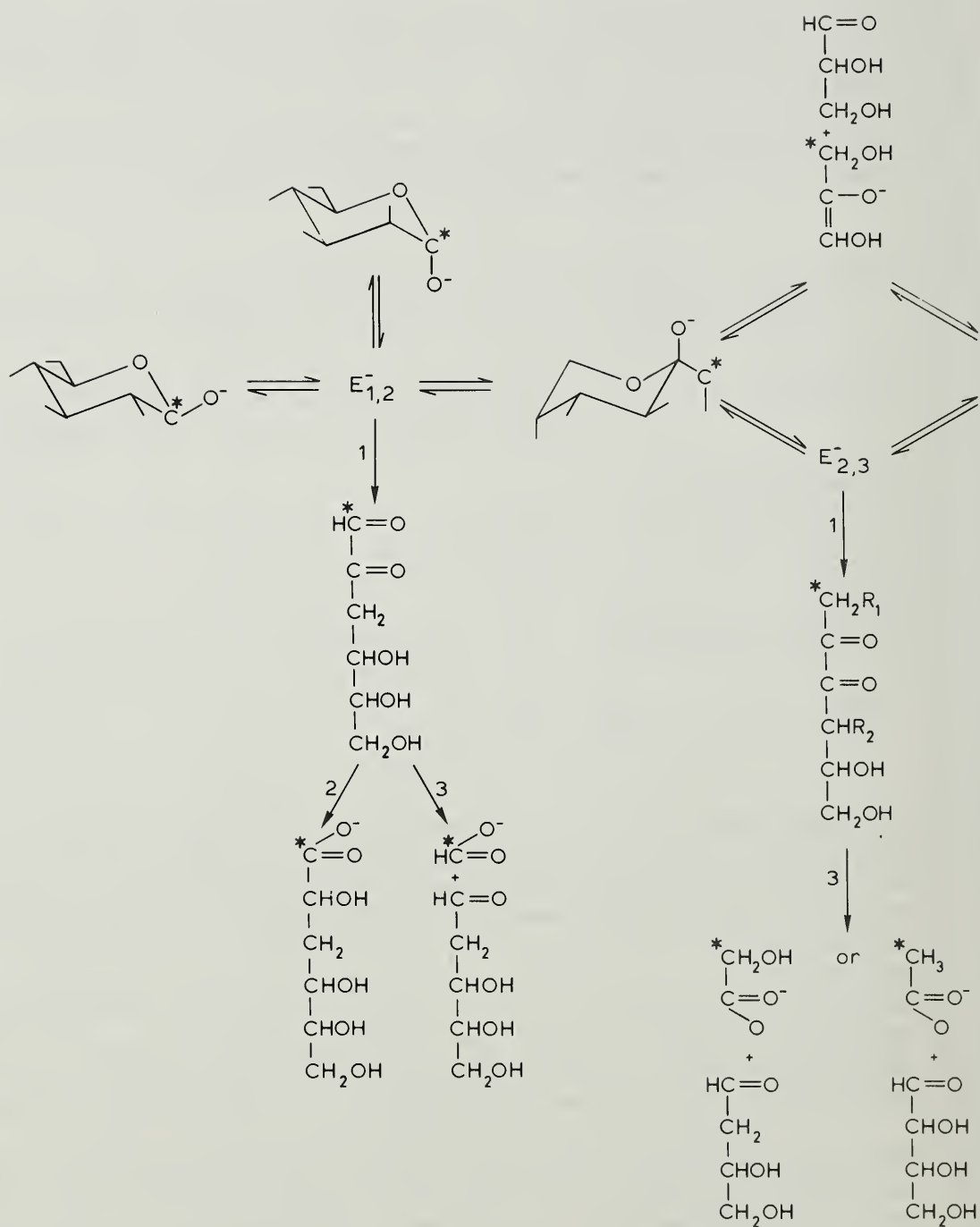
Table 1.-- ^{13}C Distribution over the functional groups in ^{13}C labeled $\text{C}_1\text{-C}_6$ acids, originating from alkaline degraded $1\text{-}^{13}\text{C}$ -D-glucose¹.

Carboxylate	Distribution of ^{13}C label (%)						
	COO-	CHOH	CH ₂	CHOH	CHOH	CH ₂ OH	CH ₃
Metasaccharinate	87	0	0	9	3	1	
Glycolate	27					73	
Lactate	41	4					55
Formate	100						
Acetate	17						83
2,4-Dihydroxybutyrate	21	0	10			69	

¹Reaction conditions: 0.025 M $1\text{-}^{13}\text{C}$ -D-glucose, 0.01 M KOH, H_2O , 78 $^\circ\text{C}$, N_2 , 7 h, 100% conversion.

Although the relatively high proportion ^{13}C label in the carboxylate group of metasaccharinic acid is evident, the presence of ^{13}C at other positions points to retro-aldolization of the monosaccharides and subsequent recombination of fragments. The almost equal ^{13}C content at C_1 and C_3 in lactic acid can be explained by $\text{C}_3\text{-C}_4$ cleavage of hexoses, subsequent isomerization of the resulting trioses, and β -elimination of the intermediate enediol anion species. The data confirm those of Sowden and Pohlen (1958) obtained for the ^{14}C distribution in lactic acid formed by alkaline degradation of $3\text{-}^{14}\text{C}$ -D-glyceraldehyde.

The relatively high ^{13}C label content of C_2 of glycolic acid and acetic acid is due to dicarbonyl cleavage of $1\text{-}^{13}\text{C}$ -4-deoxy- and $1\text{-}^{13}\text{C}$ -1-deoxyhexo-2,3-diulose, respectively. Benzilic acid rearrangement of these dicarbonyl intermediates does not occur since branched saccharinic acids have not been detected. Finally, distribution of the ^{13}C label in 2,4-dihydroxybutyric acid indicates aldolization of formaldehyde, either labeled or unlabeled, with a triose or pyruvaldehyde, either unlabeled or



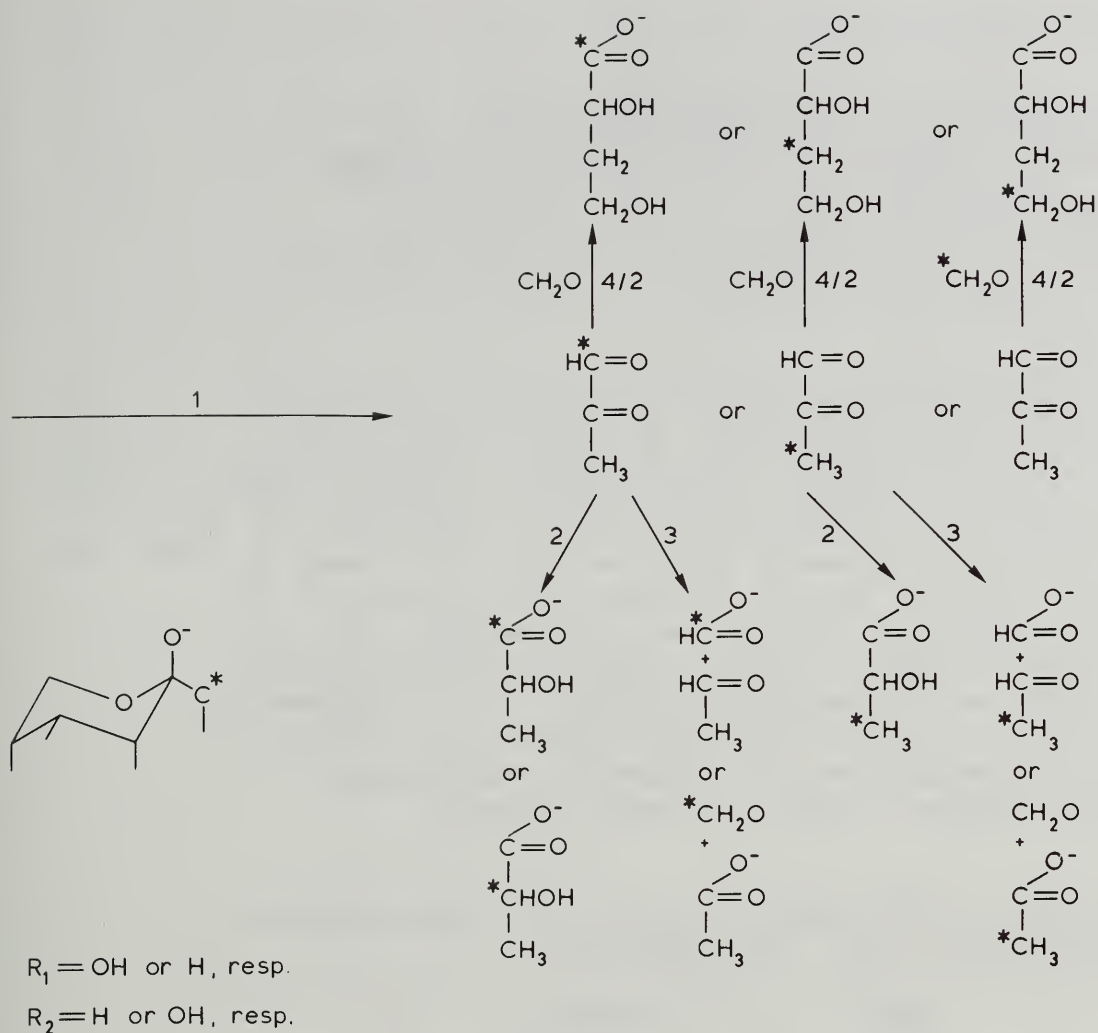


Figure 1.-Reaction scheme of the alkaline degradation of monosaccharides towards $\leq C_6$ acids as indicated by ^{13}C NMR (0.025-M 1- ^{13}C -D-glucose, 0.01-M KOH, H_2O , 78 $^\circ\text{C}$, N_2 , 7 h, 100% conversion); * denotes the ^{13}C label.

Numbering of reaction types:

1. β -elimination,
2. benzilic acid rearrangement,
3. α -dicarbonyl cleavage,
4. aldolization.

labeled at C₁ or C₃ (see above).

As the retro-aldolization of hexoses, particularly that of ketoses, into two trioses is an important reaction step, the unlabeled C₄-C₆ and labeled C₁-C₃ moieties partly undergo comparable transformations into acids. The formation of metasaccharinic acid from hexoses formed by recombination of two trioses explains the ¹³C labeling at C₄ and C₆, 9% and 1%, respectively, of this acid product.

Assuming that metasaccharinic acid contains on the average one ¹³C labeled carbon atom per molecule, which seems reasonable, the amounts of other ¹³C labeled \leq C₆ acids have been obtained from the ¹³C integrals (Table 2). In this way, it appears that 67% of the ¹³C label is incorporated in the \leq C₆ acid part of the product. This is in agreement with the total ¹³C amount in the $>$ C₆ acidic products, 32%, as determined by integration of the many small signals distributed over the spectrum. The formation of these oligomeric acidic products by aldolization of various (un)labeled (di)carbonyl compounds is too complex to be included in such a "simple" reaction sequence as depicted in Figure 1, but the coupling and rearrangement reactions are considered to be similar to the \leq C₆ region network.

Table 2.--HPLC and ¹³C NMR analysis of the \leq C₆ acids formed by alkaline degradation of 1-¹³C-D-glucose¹.

Carboxylate	HPLC Total (mol-%) ²	¹³ C NMR ¹³ C labeled (mol-%) ²	¹³ C label per molecule
Metasaccharinate	21.5	21.5	1.0
Glycolate	15.0	6.3	0.42
Lactate	17.0	10.9	0.64
Formate	24.0	5.4	0.23
Acetate	31.5	16.8	0.53
2,4-Dihydroxybutyrate	12.0	6.0	0.50

¹Reaction conditions: 0.025 M 1-¹³C-D-glucose, 0.01 M KOH, H₂O, 78 °C, N₂, 7 h, 100% conversion.

²Mol-% = mol of degradation product formed per mol of hexose, multiplied by 100%.

Simplified Overall Reaction Scheme

From recent investigations, as mentioned in the introduction, it became apparent that (i) α -dicarbonyl compounds have to be considered as important intermediates in the alkaline degradation of monosaccharides (De Bruijn et al. 1986) since conversion of these compounds by benzylic acid rearrangement, α -dicarbonyl cleavage and aldolization reactions, largely

1. isomerization via enediol anion species,
2. retro-aldolization of, in particular, ketoses,
3. aldolization of trioses,
4. enolization and β -elimination into (a) C_6 α -dicarbonyls and (b) pyruvaldehyde,
5. benzilic acid rearrangement,
6. α -dicarbonyl cleavage, leading to an acid and an aldehyde,
7. (retro-)aldolization of (di)carbonyl compounds, which is terminated by benzilic acid rearrangement (5) or α -dicarbonyl cleavage (6) of subsequent $> C_6$ α -dicarbonyl intermediates.

Also the ratio of benzilic acid rearrangement (5), α -dicarbonyl cleavage (6), and aldolization (7), the three reactions by which all α -dicarbonyls are converted, can be influenced by variation of the reaction conditions, i.e. initial monosaccharide concentration, HO^- concentration, valency of cation. Direct benzilic acid rearrangement and α -dicarbonyl cleavage of the $\leq \text{C}_6$ α -dicarbonyls result in the formation of the $\leq \text{C}_6$ acidic products. In the latter case, the reaction provides a final product (acid) as well as a still reactive aldehyde which further will undergo aldolization. The share of both benzilic acid rearrangement and α -dicarbonyl cleavage in the $\leq \text{C}_6$ acid formation is nearly constant up to $[\text{HO}^-] = 10^{-2} \text{ M}$. At higher HO^- concentrations, however, benzilic acid rearrangement occurs preferentially and lactic acid and saccharinic acids preponderate (De Bruijn et al. 1986).

The precursors for $> \text{C}_6$ acids are assumed to be formed by aldolization of α -dicarbonyls with other (di)carbonyls present

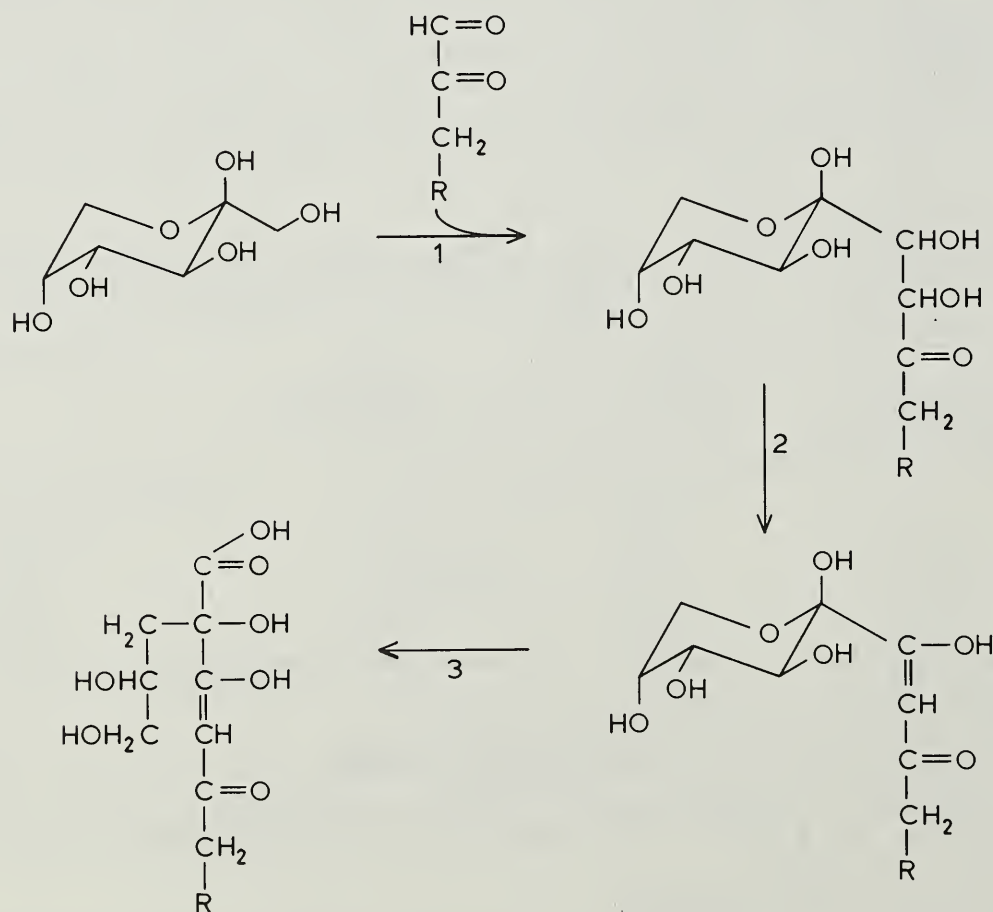


Figure 3.--Proposed mechanism for the formation of a dimeric acidic product ($\text{R} = (\text{CHOH})_2\text{CH}_2\text{OH}$).

1. aldolization,
2. enolization and β -elimination,
3. enolization, β -elimination, and benzilic acid rearrangement.

in the reaction mixture. This oligomerization is terminated by benzilic acid rearrangement or α -dicarbonyl cleavage of subsequent $> C_6$ α -dicarbonyl intermediates into $> C_6$ acids. Obviously, the latter reaction contributes to the formation of $\leq C_6$ acid products as well. In Figure 3 an example is given for the possible formation of a dimeric acidic product by such a reaction sequence. The structure as well as the molecular weight of this dimeric product corresponds to the description of oligomeric acidic products, formed by alkaline "degradation" of monosaccharides (De Bruijn 1986). Up to 50% of $> C_6$ acids may be formed at favourable aldolization reaction conditions (De Bruijn et al. 1986), i.e. moderate HO^- concentration (10^{-3} – 10^{-2} M) in combination with high monosaccharide concentrations ($> 10^{-2}$ M).

Remarks on the Degradation Behaviour of α -Dicarbonyl Compounds

Apart from enediol anion species, α -dicarbonyl compounds appear to be important intermediates in the alkaline degradation of monosaccharides. Knowledge of their molecular structure is necessary to explain the observed degradation behaviour of these compounds. In this respect, Anet (1960) established that 3-deoxyhexos-2-ulose exists in at least three forms, presumably hydrated and/or hemiacetal structures. In aqueous solution the extent of hydration of carbonyls is sensitive to the inductive effect of the substituent at the adjacent carbon atom (Bell 1966, Angyal 1984), the length (and nature) of the carbon chain (Angyal and Wheen 1980), the temperature (Angyal 1984, Angyal and Wheen 1980), and the concentration (Bell 1966). In particular, aldehydes are considerably hydrated into the aldehydrol, i.e. gem-diol, form. For example, in water at ambient temperature, formaldehyde is for $> 99\%$ present as methylene glycol (Bell 1966) and the monomers of glycolaldehyde (Collins and George 1971) and glyceraldehyde (Angyal and Wheen 1980) are hydrated to an extent of 95%. On the other hand, ketones are just slightly hydrated, if at all (Bell 1966, Angyal et al. 1976), depending on the substituents at the carbon atoms vicinal to the carbonyl groups (Angyal 1984). Besides the aldehyde/aldehydrol equilibrium, short chain sugar aldehydes, like all α - and β -hydroxyaldehydes, are also in equilibrium with dimeric forms (Angyal and Wheen 1980) which content increases at increasing concentration. Aldoses with a secondary 4-OH are almost completely present in cyclic hemiacetal forms (Angyal and Wheen 1980). In conclusion, on the analogy of hexoses, the C_6 α -dicarbonyls involved in the alkaline degradation of monosaccharides will be mainly present in (hydrated) acyclic and hemiacetal structures. Furthermore, the aldehydrol content of 1,2-dicarbonyls is supposed to exceed by far the degree of hydration of the keto groups in 2,3-dicarbonyls.

Based on these realistic structural properties of the α -dicarbonyl compounds some remarks will be made on their

degradation behaviour, as determined by the analysis of final alkaline degradation product mixtures (De Bruijn et al. 1986, Machell and Richards 1960, Anet 1964, Rowell and Green 1970, De Wit 1979). As no data are available on the relative amounts and reactivity of the different α -dicarbonyl structures, one must realize that these remarks will have a somewhat speculative character. Nevertheless, the key-role of α -dicarbonyl intermediates in the alkaline degradation of monosaccharides deserves this attention and, consequently, may induce further research in order to elucidate the striking behaviour of these compounds in aqueous alkaline solution.

As an example the structures of 3-deoxy-D-hexos-2-ulose, on the analogy of D-fructose, are depicted in Figure 4. Stereoselective benzilic acid rearrangement of this 1,2-dicarbonyl into β -metasaccharinic ($\beta/\alpha > 4$) (Machell and Richards 1960, Anet 1964, Rowell and Green 1970, De Wit 1979), having the (2S)-configuration, occurred using up to 0.1 M NaOH or KOH. This stereospecificity cannot be easily understood by enantiomeric induction in the acyclic form on the hydride shift from C₁ to C₂. The hemiacetal structures, on the other hand, contain a chiral centre at (the anomeric) C₂ to which the hydride is added in the reaction. An S_Ni-type hydride transfer with inversion of configuration at C₂ for both the β -furanose and the β -pyranose forms, which may be expected to occur predominantly on the analogy of the favourable β -conformations of D-fructose, explains the observed diastereoselective formation of β -metasaccharinic acid (Figure 5). It will be evident that in this way the α -furanose form, present in minor amount, rearranges into α -metasaccharinic acid.

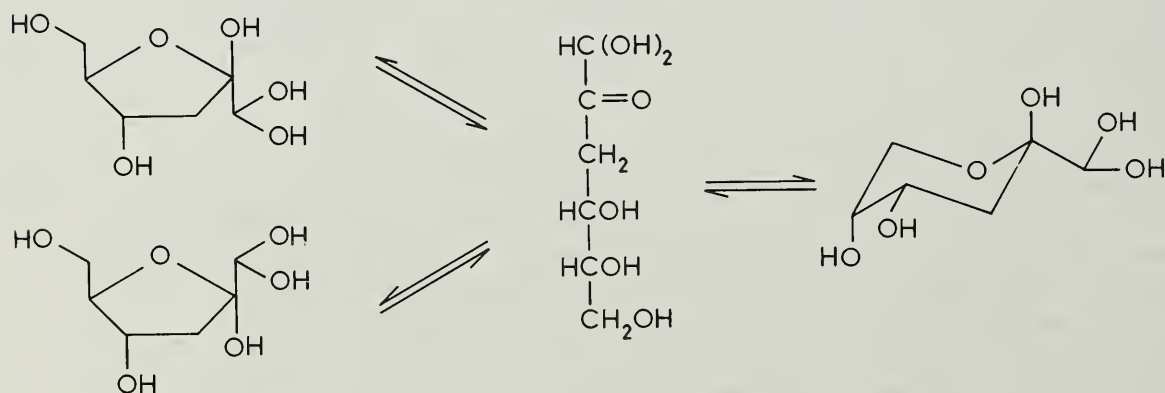


Figure 4.--Most probable acyclic and cyclic structures of 3-deoxy-D-hexos-2-ulose (β -pyranose $>$ β -furanose $>$ α -furanose on the analogy of D-fructose).

The alkaline degradation of 2,3-dicarbonyls is quite different from that of the 1,2-dicarbonyl compounds as illustrated by our alkaline degradation experiment with 1-¹³C-D-glucose. As shown

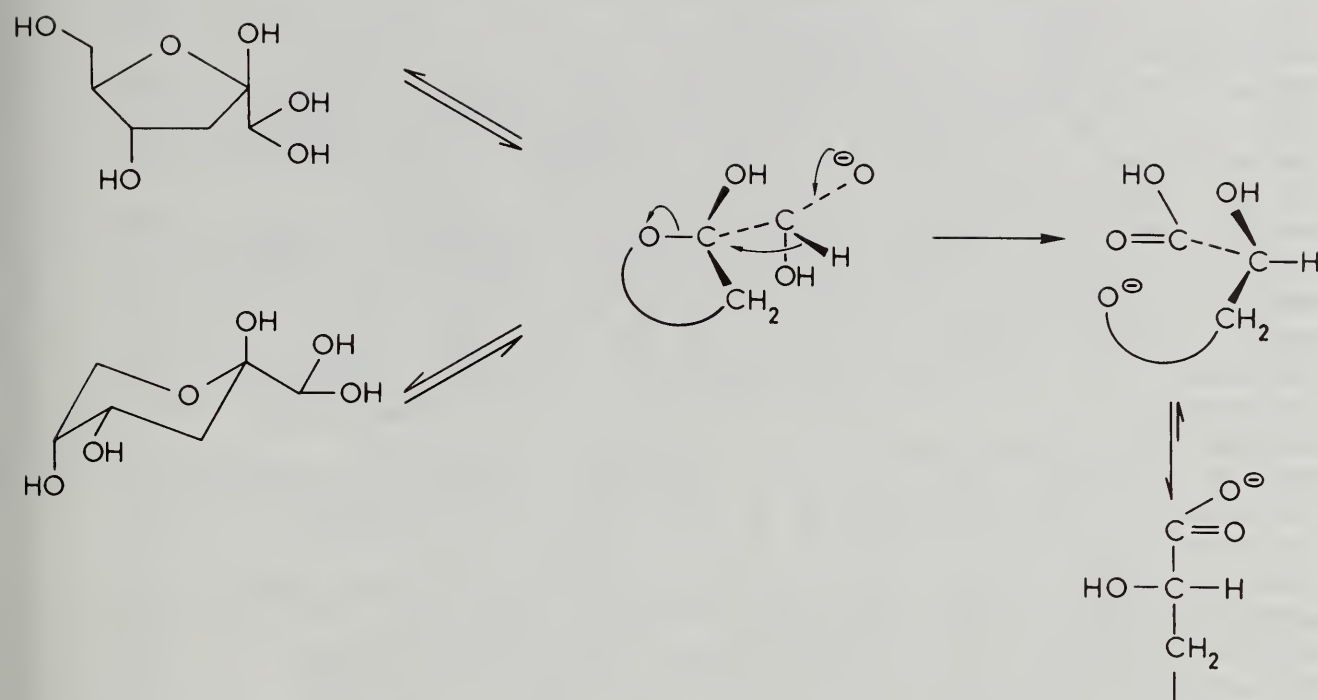


Figure 5.--Stereoselective benzilic acid rearrangement of the β -furanose and β -pyranose form of 3-deoxy-D-hexos-2-ulose into β -metasaccharinic acid.

by ^{13}C NMR spectroscopy no isosaccharinic acid and saccharinic acid were formed by degradation of 0.025 M 1- ^{13}C -D-glucose in 0.01 M KOH (H_2O , 78 $^\circ\text{C}$, N_2 , 7 h, 100% conversion), but only typical α -dicarbonyl cleavage products of the 2,3-dicarbonyl intermediates, i.e. glycolic acid and acetic acid, could be determined in the final reaction mixture. On the other hand, upon addition of calcium(II) benzilic acid rearrangement of 2,3-dicarbonyls was established by the observation of substantial amounts of isosaccharinic acid and saccharinic acid as well as branched C_4 and C_5 saccharinic acids (De Bruijn et al. 1986). The different degradation behaviour of 2,3-dicarbonyls in comparison with that of 1,2-dicarbonyls might be due to the lower degree of hydration of the keto groups and the fact that a shift of a CH_3 or CH_2OH carban ion instead of a hydride is involved in the benzilic acid rearrangement.

The effect of the HO^- concentration on the benzilic acid rearrangement/ α -dicarbonyl cleavage/aldolization ratio of α -dicarbonyl intermediates can be understood considering their ionization behaviour. The α -dicarbonyls will have a pK_a of 10-11 as estimated from $\text{pK}_a = 11.0$ for pyruvaldehyde and $\text{pK}_a = 10.3$ for 1,2-cyclohexanedione (Serjeant and Dempsey 1979). Furthermore, a second ionization may occur at $[\text{HO}^-] > 10^{-1} \text{ M}$ as expected from the pK_a 's of 1,2-dihydroxybenzene ($\text{pK}_1 = 9.45$,

$pK_2 = 12.8$) (Serjeant and Dempsey 1979) and of hydrated carbonyl compounds like formaldehyde ($pK = 13.27$) (Serjeant and Dempsey 1979), acetaldehyde ($pK = 13.57$) (Serjeant and Dempsey 1979), and monosaccharides ($pK^a \sim 13$) (De Bruijn et al. 1986). Using $pK_1 = 10$ and $pK_2 = 13$, in Figure 6 the neutral and ionized forms of α -dicarbonyls are depicted as a function of the HO^- concentration. The neutral species will undergo benzilic acid rearrangement and α -dicarbonyl cleavage. As the enolate, required for aldolization, is not present only a small amount of oligomeric products is formed. The ionized (enol) species ($10^{-4} M < [HO^-] < 10^{-1} M$), on the other hand, is responsible for a substantial formation of oligomeric products. The benzilic acid rearrangement of α -dicarbonyls (Selman and Eastham 1960), in contrast to their hydrolytic cleavage, depends on the HO^- concentration. Ionization markedly increases the benzilic acid rearrangement at the cost of the cleavage reaction, especially at $[HO^-] > 10^{-1} M$, because of the promoting effect of ionization on the shift of R_1 to the other carbonyl group. As the formation of oligomeric products involves aldolization reactions between neutral and ionized (di)carbonyl compounds (De Bruijn et al. 1986), it will be obvious that at high alkalinity ($[HO^-] > 10^{-1} M$), when almost all carbonyls are ionized, this reaction is of little importance in the product formation.

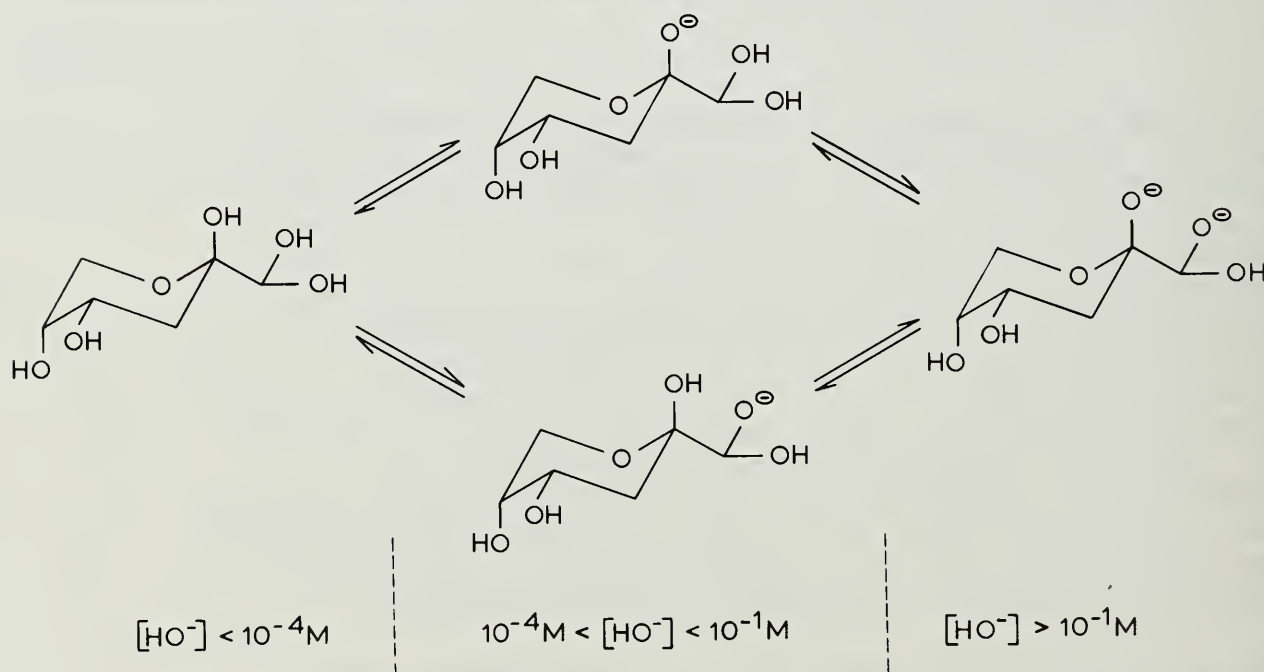


Figure 6.--Ionization of the β -pyranose form of 3-deoxy-D-hexos-2-ulose as a function of the HO^- concentration.

CONCLUSIONS

Enediol anions are the well-known intermediates in the isomerization of monosaccharides as well as the starting intermediates in the subsequent alkaline degradation.

Monosaccharides themselves, in particular ketoses like D-fructose and D-psicose, may be considered too as intermediates in the alkaline degradation reaction by their retro-aldolization into small saccharides (and vice versa). The extent of retro-aldolization of monosaccharides appears to depend strongly on the HO^- concentration.

Considering α -dicarbonyl compounds, i.e. the β -elimination products of enediol anion species, as key-intermediates completes the mechanistic picture of the alkaline degradation of monosaccharides. The influence of reaction parameters on the benzilic acid rearrangement/ α -dicarbonyl cleavage/aldolization ratio of these intermediates, as determined by the change of the final product composition, can be understood by the observed effects of these parameters on the reactivity of the various (hydrated) acyclic and hemiacetal α -dicarbonyl structures involved.

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DISCUSSION

Joseph A. Polack, Audubon Sugar Institute - With respect to those principal pathways, would there be any advantage to try to shift product distribution towards the higher molecular weight C-6 acids? If so, what are the chances of doing that?

Kieboom - Yes, there can be some advantage in obtaining more of the polymeric products, especially when that is at the cost of lactic acid formation. The lime use is reduced from 2 to 1.4. Calcium hydroxide does two things: the right and the wrong thing. The right thing is that it produces substantial amounts of polymeric material; the wrong thing is that the calcium ions split up fructose in such a way as to produce large amounts of lactic acid, which we don't want. It would be nice to have a monovalent cation with the same property as calcium, i.e. one that would give insoluble salts. That's a wish, not a solution. It's quite difficult to choose the best way.

Another point is that in the industrial practice we also must consider the Maillard reaction. The fifth variable is amino acid or protein content; its influence should be included for the total picture.

Margaret A. Clarke, S.P.R.I. - I have two questions arising from this elegant work: the first concerns formaldehyde, still widely used, especially outside the U.S., for microbiological control in the diffuser. What reactions would you anticipate with formaldehyde in limed juice?

Kieboom - This is a topic discussed for many years. It's always been stated that the formose reaction starting from formaldehyde and the alkaline degradation starting from the C-6 sugars are principally different and have nothing to do with one another. We were interested to see the interference of formaldehyde if it is present in an alkaline degradation mixture. The diagrams in the attached figure show variations in the final product composition going from the formose reaction towards the alkaline degradation reaction under similar conditions. Formaldehyde, after all, is just a C-1 sugar. We have done the variation, going from pure fructose to pure formaldehyde, under the same reaction conditions, with calcium hydroxide as base. We've looked in particular at the formation of polymeric products. There are two important points: the total reaction mixture from the formose reaction is the same as from alkaline degradation--it's the same principle. Surprisingly, there is a comparable formation of C-6 polymeric acids from formaldehyde and fructose. Under practical factory conditions, the products found from formaldehyde are similar to the products from sugar. So, the use of formaldehyde will not create any compounds other than those that would be formed by alkaline decomposition of sugar.

Clarke - My second question concerns the synthetic possibilities. Are any of the oligomeric C-6 acids, for example, useful as industrial products? Could these reactions be employed as a synthetic route from sugar?

Kieboom - Simple treatment of glucose with alkali gives a polymeric product in 50% yield. These polycarboxylates might have good sequestrant and complexation properties, but we haven't tested the sugar degradation products for this yet. The price of these products should not be very high.

N. W. Broughton, British Sugar plc - A comment on the degradation, or polymerization, of formaldehyde, compared with the reactions of fructose. In the 1960's at British Sugar, a lot of work was done on this topic. Our findings agreed with what you've just said. We used, at that time, thin-layer chromatography, and were able to show that in laboratory carbonatation systems, the products from formaldehyde were identical with those from fructose breakdown. I don't think that this necessarily means no problem from formaldehyde, because formaldehyde is used in a large shock dose, so that the peak concentrations can be many times (several hundred times) the concentration taken as a weight average. We have data indicating that a shock dose of formaldehyde can create a wave of color (I don't know about the acids) moving through the process.

Kieboom - I think the color formation leads us into the Maillard reaction, a variable that has not been studied by us. Apart from this, a high formaldehyde/fructose ratio doesn't principally affect the final product composition as is shown in the attached figure.

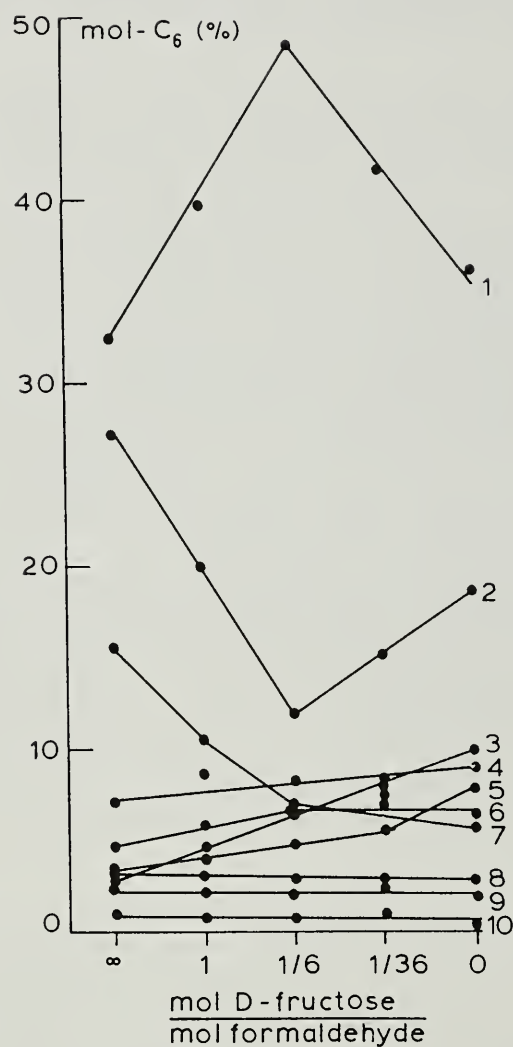


Fig. 1. Carboxylic acid products as a function of the molar D-fructose/formaldehyde ratio. Reaction conditions: 0.025 M D-fructose (except at the ratios 1/36 (0.0042 M) and 0 (0.15 M formaldehyde)); 590 mg Ca(OH)₂ in 100 ml H₂O, 78 °C, N₂, 7 h, 100% conversion.

- | | |
|------------------------------|--------------------------------------|
| 1. > C ₆ acids | 6. C ₅ -saccharinic acids |
| 2. lactic acid | 7. C ₆ -saccharinic acids |
| 3. 2-methylglyceric acid | 8. acetic acid |
| 4. 2,4-dihydroxybutyric acid | 9. glycolic acid |
| 5. formic acid | 10. glyceric acid |

(J.M. de Bruijn, A.P.G. Kieboom, and H. van Bekkum, J. Carbohydr. Chem., 5, 1986, 561-569.)

THE PRODUCTION OF LIQUID INVERT SUGAR WITH ION EXCHANGE RESINS

R. W. Percival and J. E. Schuler

Rohm and Haas Company, Latin American Region

INTRODUCTION

The production of liquid invert sugar is of growing interest in certain regions of the world such as Latin America and the Caribbean as a result of the increasing demand for "industrial" liquid sugar by the bottling, candy, baking and other industries. Because of freight and duty restrictions, the inversion of sucrose, in many cases, is the process of choice because of the availability of local cane sugar resources. The purpose of this paper is to review the application of ion exchange technology in the areas of inversion, ash removal and decolorization in the production of liquid invert sugar. The advantages of the ion exchange processes versus the conventional homogeneous inversion with mineral acid will also be discussed.

EXPERIMENTAL AND ANALYTICAL

The homogeneous acid inversion experiments were carried out in a 3 liter round bottom flask equipped with condenser, stirring shaft and paddle, stirring motor, heating mantle and voltage regulator. The desired temperature was maintained using a pot-lifter and thermo-watch temperature regulator. The syrups were adjusted to the desired pH (1.8) by the dropwise addition of concentrated hydrochloric acid.

The neutralization with caustic soda was accomplished using sodium hydroxide (approximately 1 normal) added dropwise to an aliquot of the inverted syrup until the desired pH was attained (5.0-5.5).

Neutralization with the weak base resin was accomplished by passing the acid inverted syrup through a 50 ml bed of Duolite A-392S (OH) in a jacketed, 2 ft. high, 1/2 inch (inner diameter) glass column maintained at the desired temperature by means of a constant temperature circulating bath. The flow rate of choice was maintained using a Milton Roy Minipump.

The ion exchange process of decationization followed by inversion and then neutralization consisted of three columns hooked up in series. The columns were glass, jacketed, 1/2 inch (inner diameter) and 2 ft. high.

The first column contained 50 ml of Amberlite IR-120 (H) followed by a column containing 50 ml of Amberlite IR-118 (H). The last column in the series contained 50 ml of Duolite A-392S (OH). The temperature and flow rate were maintained as described in the previous paragraph.

All pH values were obtained using an Orion Model 611 pH meter.

All Brix determinations were carried out with an Abbe Mark II digital refractometer.

The ash values reported in this paper were conductivity ash determined with a YSI Model 31 conductivity bridge.

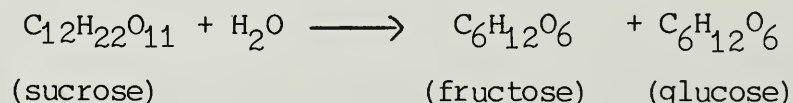
Syrup color values were measured at pH 7 and 420 nm on a Hach DR/3 spectrophotometer.

The HMF values were obtained using a Perkin Elmer Lambda 3B spectrophotometer.

RESULTS AND DISCUSSION

Homogeneous Inversion

Catalysis of the inversion of sucrose by the addition of a soluble mineral acid is termed homogeneous catalysis and has been well understood. Sugar inversion is defined as the hydrolysis of sucrose, a disaccharide, to form invert sugar, an equimolar mixture of fructose and glucose, two monosaccharides:



Many sugar mills and refineries in Latin America produce invert syrup by the homogeneous acid process and neutralize the acid with caustic soda at the completion of the inversion step. The neutralization of acid with caustic soda results in the formation of an equivalent amount of ash. The amount of ash formed by this reaction may result in an ash level beyond the bottling standard for ash in a liquid sugar. In our discussions with sugar companies, interest has been expressed in the benefits of ash level control and the color removal that would be realized through the use of a weak base anion exchange resin as a substitute for the use of the soluble caustic soda to neutralize the acid used for the

inversion. Our laboratory studies have successfully demonstrated that a weak base resin (Amberlite IRA-93 or Duolite A-392S) can be used as a substitute for caustic soda to neutralize the acid without forming ash and also partially decolorize the sugar syrup as demonstrated by the following data:

A. Brazil syrup (remelted and clarified Brazilian raw sugar) inverted with HCl at pH 1.8 and temperature = 70° C for 4 hours (99% inversion).

1. Inverted syrup neutralized with NaOH.

Final pH	= 5.1
Brix	= 59.3
Color	= 189 mau
Conductivity ash	= 2579 ppm
HMF	= 344 ppm

2. Inverted syrup neutralized by ion exchange in the column mode, i.e., Duolite A-392S at 4 bv/hr and 40° C.

Final pH	= 8.1
Brix	= 59.7
Color	= 30 mau
Conductivity ash	= 509 ppm
HMF	= 46 ppm

B. Brazil syrup inverted with HCl at pH 1.8 and temperature = 40° C for 10 hours (55% inversion).

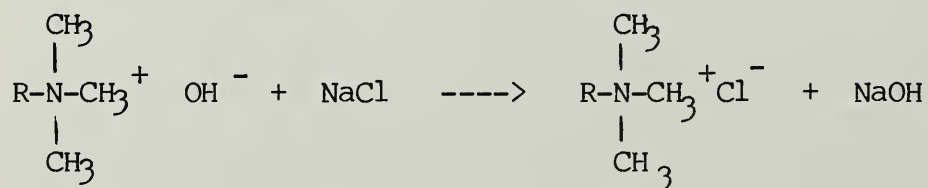
1. Inverted syrup neutralized with NaOH.

Final pH	= 5.3
Brix	= 58.4
Color	= 121 mau
Conductivity ash	= 2046 ppm
HMF	= 19 ppm

2. Inverted syrup neutralized with Duolite A-392S at 4 bv/hr and 40° C.

Final pH	= 8.9
Brix	= 58.4
Color	= 9 mau
Conductivity ash	= 552 ppm
HMF	= 9 ppm

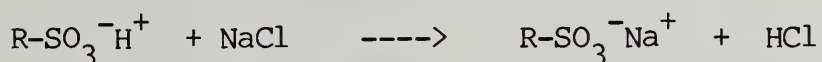
The above data demonstrate that under the conditions studied the ion exchange neutralized system produces a significantly higher quality liquid invert syrup as determined by color, ash and hydroxymethylfurfural (HMF). The higher pH values for the ion exchange system are due to the ash in the original syrup reacting with the small number of strong base sites present in the weak base resin during the initial stage of the operating cycle.



Should this higher pH represent a problem with respect to bottling standards one has a couple of corrective actions which could be considered, for example, deashing the original syrup, or a regenerant that will not activate the strong base sites (e.g., ammonium hydroxide or soda ash).

Heterogeneous Inversion by Ion Exchange

It is common practice to use cation exchange resins to invert sucrose. If the sucrose syrup is not completely free of ash, the electrolytes present in the syrup will generate soluble acidity by the reaction of the cation exchange resin (in the acid form) with the ash present in the syrup.



The liberated free mineral acidity will of course serve as an excellent catalyst for the homogeneous inversion of sucrose. The amount or concentration of the acidity generated will depend on the ash level in the sugar. The inversion of sucrose may also be catalyzed by the cation exchange resin itself even in the absence of any ion exchange generated soluble acid. Therefore, it is common practice to either deash the syrup by means of an ion exchange mixed bed if the ash content is low (100 ppm or less) or to use a cation exchange resin column (Amberlite IR-120 or Duolite C-20) ahead of the cation exchange inversion resin (Amberlite IR-118 or Duolite C-291) to decationize the syrup and protect the inversion resin against exhaustion. The primary cation exchange column is then regenerated with sulfuric or hydrochloric acid after exhaustion to the salt form. The following data not only serves as an example of heterogeneous inversion by ion exchange, but also demonstrates the effect of flow rate on the degree of inversion:

Column Runs: 50 ml bed of Amberlite 120(H) followed by a 50 ml bed of Amberlite 118 (H) maintained at 40° C. The syrup influent with a clarified 60 Brix Brazilian raw sugar having a color of 122 mau and 719 ppm ash.

<u>Flow Rate</u> <u>bv/hr</u>	<u>%</u> <u>Invert</u>	<u>Color, mau</u>	<u>pH</u>
1.2	87	92	2.0
2.0	79	73	2.1
3.0	72	73	2.0
4.0	57	73	2.0

Inversion, Deashing and Decolorization

Many ion exchange inversion systems now utilize a weak base anion exchange resin (e.g., Amberlite IRA-93 or Duolite A-392S) operated in series with the cation exchange resin and catalytic inversion resin to neutralize the acidity formed by the salt splitting reaction of the cation exchange resin with the ash in the syrup.

As can be seen by the data presented below, the higher porous weak base anion exchange resin not only neutralizes the acidity and lowers the ash level but also removes a substantial portion of the organic acid color bodies. The ion exchange system thereby provides a means of achieving inversion, ash removal and decolorization in a single pass through the resin.

A. Complete Resin System: Amberlite Amberlite Duolite
 IR-120 IR-118 A392S

1. Refined cane sugar syrup influent (60.4 Brix, pH 7.2, color = 35 mau, ash = 154 ppm, HMF = 4 ppm).

Flow Rate bv/hr	Temp. C	Brix	pH	Color mau	Ash ppm	HMF ppm	% Invert
1.2	40	61.8	4.9	16	4	21	87
1.2	50	61.0	3.8	51	13	322	96
4.0	40	61.7	4.8	7	5	29	57

2. Clarified Brazilian raw sugar syrup influent (58.0 Brix, pH 7.9, color = 144 mau, ash = 719 ppm, HMF = 15 ppm).

Flow Rate bv/hr	Temp. C	Brix	pH	Color mau	Ash ppm	HMF ppm	% Invert
1.2	40	60.1	4.2	11	6	53	87
1.2	50	59.7	4.6	21	10	110	96
4.0	40	60.4	4.4	3	7	24	57

The purpose of this laboratory study was to compare the quality of liquid invert sugar produced by homogeneous acid catalysis and neutralization with caustic soda versus acid neutralization with a weak base resin. The inversion, deashing and decolorization of sugar by means of an ion exchange process was also evaluated using a remelted refined sugar as well as a remelted and clarified Brazilian raw sugar.

CONCLUSIONS

Ion exchange resins can be used to invert sucrose and to produce liquid invert syrups containing very low levels of ash, color and HMF. Weak base anion exchange resins can be used as a substitute for caustic soda in the neutralization of mineral acid used in the

inversion process to reduce the ash and HMF content of the invert syrup and to achieve a reduction in color in the final product.

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DISCUSSION

Joseph F. Dowling, Refined Sugars, Inc. - What was the advantage of putting IRA 120 in front of IRA 118, rather than using only 118?

Percival - Amberlite IR-118 can be used alone, but we put the decationizing column of Amberlite IR-120 first in order to prevent exhaustion of Amberlite IR-118 with cation components of the ash in sugar. Amberlite IR-120 has a higher ratio of crosslinking and a higher volume capacity. In order not to use up the capacity of the inversion column, we put Amberlite IR-120 in front to protect it.

X. Lancrenon, Applexion - Would you comment further on the HMF removal by the weak anion resin on one side and the HMF production when you pass through the catalytic column?

Percival - Yes; we've seen the reduction of HMF content of acid inverted syrup neutralized with resin versus acid neutralized with caustic soda in our data. The sugar inverted with acid at 70° C to 99% invert and neutralized with caustic soda produced a level of over 300 ppm HMF. When we neutralized the same sample of acid inverted sugar with Duolite A392S or Amberlite IRA-93 we detected almost one-tenth the level of HMF observed in acid inverted syrup neutralized with sodium hydroxide. So, we concluded that HMF was physically adsorbed by the anion exchange resin in the early part of an operating cycle. It may leak through later in the operating cycle. This study is still in progress. It would be extremely interesting if the weak base resin is able to physically adsorb the HMF.

Leif Ramm-Schmidt, Finnish Sugar Co. - As you change neutralization from caustic soda to resin, what is the change in chemical costs?

Percival - The chemical cost, using a weak-base resin, is only 15% higher than the sodium hydroxide cost for neutralization. That is, the resin is a weak-base resin that regenerates at about 115% of stoichiometric requirement. The operating cost is very low. For that 15% higher cost, there is the additional benefit of color removal and the prevention of formation of ash.

R. Dickey, Refined Sugars, Inc. - In your data, you showed flow rates, rates of inversion and colors. Were those colors measured at low pH?

Percival - Those colors were measured by the ICUMSA method at pH 7.

SOME LABORATORY OBSERVATIONS ON THE INVERSION OF SUCROSE WITH STRONGLY ACIDIC CATION EXCHANGE RESINS

Mark Wnukowski and C. Chi Chou

American Sugar Division
Amstar Sugar Corporation, New York

INTRODUCTION

In the sugar industry the term inversion is most frequently used to describe the rotational change following the acid hydrolysis of a sucrose solution, when the strong dextrorotation of sucrose is inverted to the levorotation of the resulting mixture of glucose and fructose or invert sugar (Chen 1985).

The study of this hydrolysis of sucrose catalysed by H^+ ions is by far no new field of endeavor to sugar technologists and chemical researchers in general. In fact work done on the inversion of sucrose by soil can be traced back to studies done by Tacke and Suchting (1911) and Hanley (1914). Furthermore, research dealing directly with the subject of this paper, namely the inversion of sucrose using cation exchange resins, can be cited as early as Bodamer and Kunin (1951) and as recently as Siegers and Martinola (1985).

Evidently an appreciable amount of work has been done in the area of invert sugar production processed by cation exchange resins. Additionally, it is common knowledge that commercial installations employing such technology have been designed and been in existence for some time now (Refined Syrups and Sugars, Inc. 1955).

In this paper some of the findings previously reported on the use of strongly acidic cation exchange resins for the inversion of sucrose are reexamined and further elaborated on. This is done in the light of our initial investigative goal, which was to determine whether a premium bottler's invert syrup meeting certain compositional specifications (i.e., low hydroxymethylfurfural content HMF) could be produced in a one-step process. This would hopefully be achieved by inverting white granulated sugar syrup with a cation exchange resin

column with no further chemical treatment. This is a departure in thought from most recommendations to date which suggest that the feed syrup should first be demineralized prior to being inverted (Siegers and Martinola 1985 and Berghoter et al. 1977).

Two types of commercially available strongly acidic cation exchange resins were chosen to be evaluated for this purpose: 1) A gel-type with a degree of cross-linking corresponding to approximately 4.0% divinylbenzene (DVB) and 2) a macroporous resin cross-linked with approximately 5.5% DVB.

MATERIALS AND METHODS

Apparatus

- a) Two different sizes of jacketed chromatographic columns were employed as vessels to hold the resin beds. The dimensions were 1) height 25.4 cm., I.D. 2.54 cm. and 2) height 50.8 cm., I.D. 2.54 cm. These columns were obtained from Kontes Scientific Glassware and Instruments.
- b) Thermostated temperature control of the columns was achieved with a Lauda/Brinkmann Refrigerated Circulation Bath model K-2/R from Brinkmann Instruments.
- c) Accurate flow was controlled by use of a Technicon Autoanalyzer Proportioning pump outfitted with the appropriate Acculab precision pump tubing obtained from Fisher Scientific.
- d) The effluents from the resin columns were collected and fractionated continuously using a LBK Rotator and Radi Rac Fraction Collector Controller types 3401B and 3403B, respectively, manufactured by LBK Instruments Inc.

Reagents

- a) 5-Hydroxymethylfurfuraldehyde standard was obtained from the Sigma Chemical Co.
- b) Both the 2N HCL and 1.5N NaOH used in the conditioning of the resin columns were prepared from J.T. Baker Analyzed Reagents diluted to concentration with demineralized water.

Experimental Methods

- a) Column Conditioning and Preparation: All resin columns both gel-type and macroporous were loaded and conditioned in the same way according to the following instructions of the resin manufacturer.

The resin was first settled in a beaker of demineralized water overnight. Swelling of dry resins in the column could cause violent rupture of the housing.

After loading the glass chromatographic column, the resin was backwashed with water to expand the bed at least 50%. This was done to remove air bubbles and arrange the particles by size. The resin was allowed to settle and the water drained leaving one inch of water above the bed. The resin was then washed with at least two bed-volumes of 2N HCL in approximately twenty minutes. The acid was then washed out with five bed-volumes of water (thirty minutes), and the residual water drained leaving one inch of water above the bed. Two bed-volumes of 1.5N NaOH were then fed, followed by a rinsing cycle of five bed-volumes of water. Again the water was drained to approximately one inch above the bed. This entire acid rinse - caustic rinse procedure was then repeated a second time.

Next the resin was treated with two additional acid rinse cycles, the only change being the length of the rinse portion of the first cycle was reduced from five to only one bed-volume of water.

- b) 5-Hydroxymethylfurfural was determined in the syrup samples by adopting a spectrophotometric procedure reported by White (1979) for HMF content in honey.

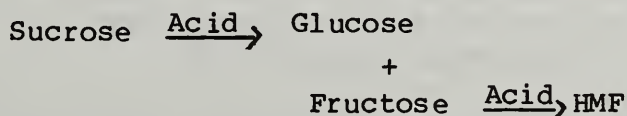
This procedure was tested on cane invert syrup samples spiked with standard HMF. Studies conducted by our laboratory found recoveries in the area of 93.0 to 99.8%.

RESULTS AND DISCUSSION

In view of the amount of work performed in the area of sucrose inversion by strongly acidic cation exchange resins, it was only logical to draw upon these facts and information in the planning and initiation of our studies.

For example, our requirements were to produce a premium grade invert syrup (94.0% invert minimum), with low color, ash and chloride concentrations, and a hydroxymethylfurfural concentration (HMF) of not more than 75 ppm.

HMF is a by-product of the acid degradation of sucrose along the fructose pathway as shown in the following reaction equation:



HMF is a yellowish compound that is difficult to remove by conventional refinery methods and gives the invert syrup an undesirable color.

This product with the above attributes was to be produced in a one-step treatment of white granulated sugar syrup with a strongly acidic cation exchange resin with no prior demineralization of syrup.

Demineralization is recommended to remove metal cations contained in the syrup which would load down the resin column and decrease the inversion efficiency. This also leads to more frequent regenerations of the resin and an earlier column death.

In view of these facts, previous work published by Siegers and Martinola (1985) was instrumental in setting the initial parameters for our experiments and resins.

Their findings indicated that for most viable acidic cation exchange resins to achieve inversion levels of 99.0% a minimum space time of about 50 minutes (flow rate 1.2 bed-volumes per hour) must be provided. The space time being defined as by Gilliland et al. (1971) as the reciprocal of the flow rate in minutes.

Additionally, to maintain HMF concentrations at their lowest the columns should be operated as close as possible to 40°C.

Using this information, four columns were set up, two long columns 5.08 cm. in height and two shorter at 25.4 cm. One of each size column was filled with a readily available commercial macroporous resin and the others with a gel-type cation exchange resin. The bead size of both resins was approximately 0.6mm, while the degrees of divinylbenzene cross-linking were approximately 4.0% for the gel and 5.5% for the macroporous. The bed-volumes of the columns were calculated geometrically and were found to be 60 cm.³ of resin for the short columns and 120 cm.³ for the longer. The flow rates to these columns were adjusted with the appropriate pump tubing to provide specific flow rates of 1.0 bed-volume per hour to each column. The columns were temperature controlled at 40°C and the feed syrup reservoir containing 60° Bx white granulated sugar syrup was kept at ambient temperature.

Analyzing the effluents from these columns for % inversion by the conventional Lane-Eynon method found neither of the resins to be producing the minimum of 94.0% invert required for our product at a specific flow rate of one bed-volume per hour (see Figure 1). The gel resin performed better than the macroporous, producing 92.1% invert versus 80.7%.

At this point, the columns were shut down, regenerated, and restarted, increasing the space time from 60 to 120 minutes or 0.5 bed-volume per hour in effort to increase the efficiencies of the resins.

Measuring the percent invert at this time it was found that the gel-resin was now yielding an acceptable level of 98.8% invert while the macroporous resin was still operating below expectations, producing invert at only 92.7% (Figure 1).

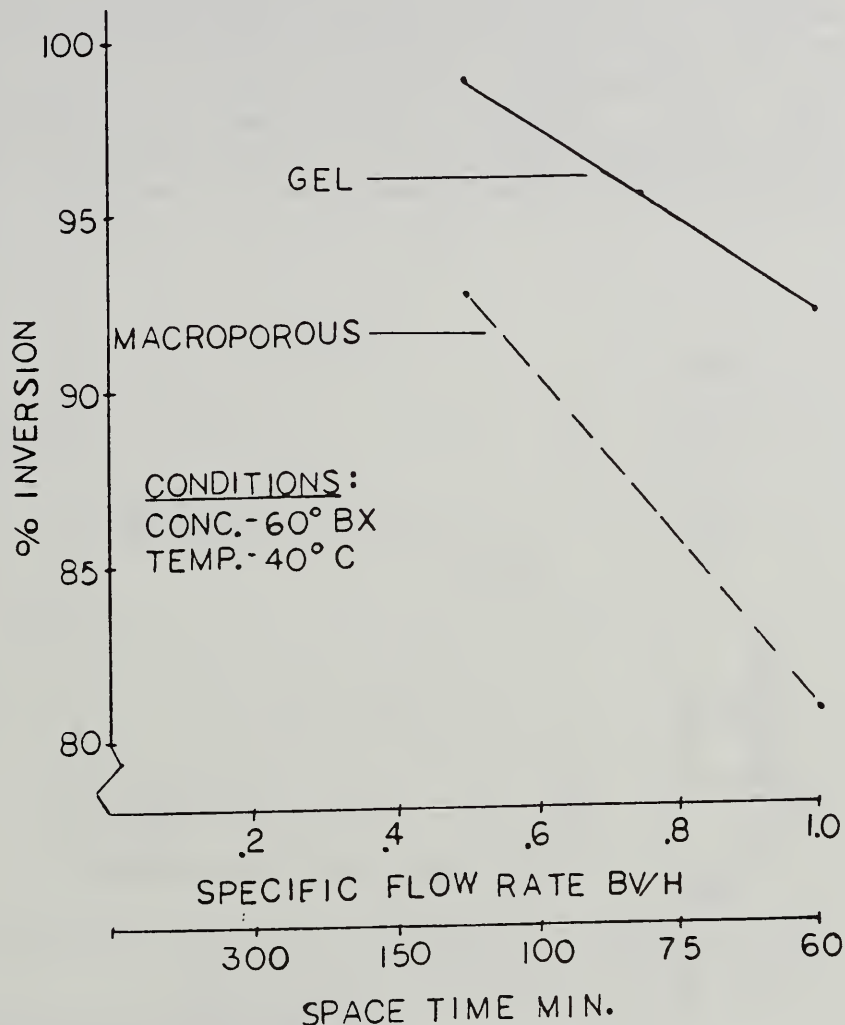


FIGURE 1.--PERCENT INVERSION VERSUS SPECIFIC FLOW AND SPACE TIME FOR THE GEL TYPE AND MACROPOROUS RESINS.

Because of the high osmotic loads that can occur during the operation of resin columns in a sugar refinery, due to the sweetening-on and sweetening-off processes as well as regeneration, it is highly desirable to employ a macroporous resin. The reasons for this are resins with a low degree of cross-linking are mechanically less strong than those with higher degrees of cross-linking. Macroporous resins are also known to have a greater mechanical strength than the gel-type resins. Additionally, macroporous resins are favored in anticipation of experiencing a significantly smaller pressure drop during operation than their gel-type counterparts.

In light of these facts, a further attempt at increasing the efficiency of the macroporous resin columns was tried. This entailed elevating the operating temperature of the columns from 40°C to 43°C and finally to a maximum of 46°C.

Analyzing the effluents from these macroporous columns (See Figure 2) for % invert found, that even at a temperature of 46°C the column with a specific flow rate of 1.0 bed-volume per hour only attained an inversion level of 87.0%. Further it was found that a temperature of at least 41.5°C was necessary to reach our minimum requirement of 94.0% invert for the macroporous resin columns operated at 0.5 bed-volume per hour.

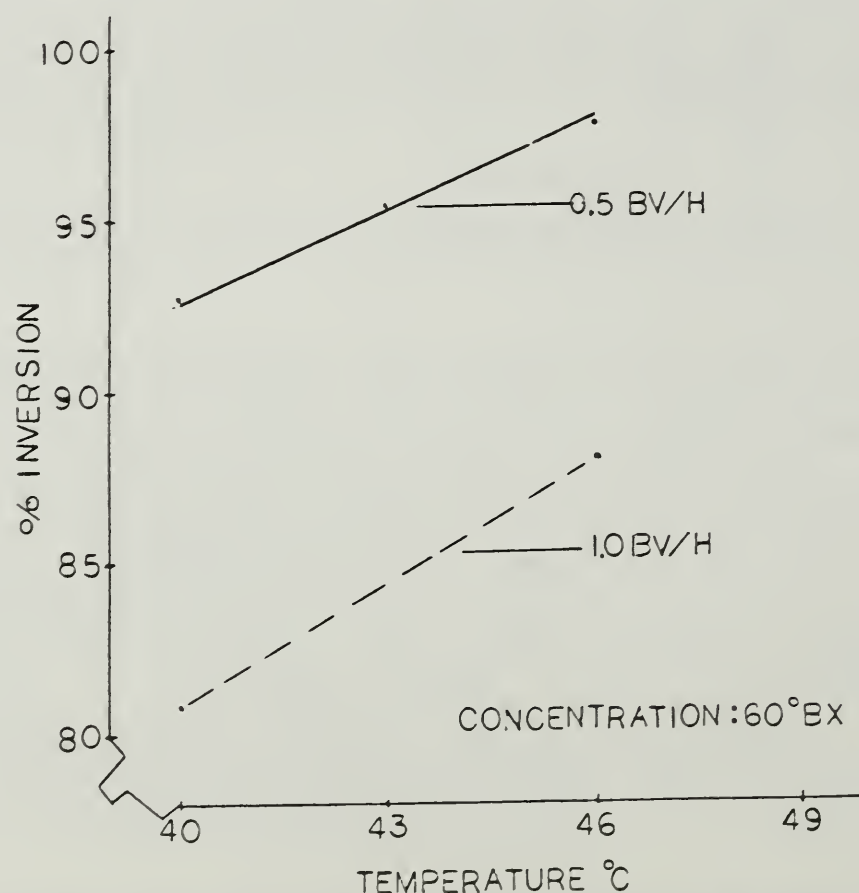


FIGURE 2.-- EFFECT OF SLIGHT CHANGES IN TEMP. ON THE PERCENT INVERSION FOR THE MACROPOROUS RESIN AT FLOW RATES OF 0.5 AND 1.0 BV/H.

Also taken into account at this portion of our study were the data generated by our HMF analyses illustrated in Figure 3. Viewing these results, it can be seen that all values of HMF concentrations for the macroporous resin across the temperature range of 40°C to 46°C exceeded our requirement of 75 ppm.

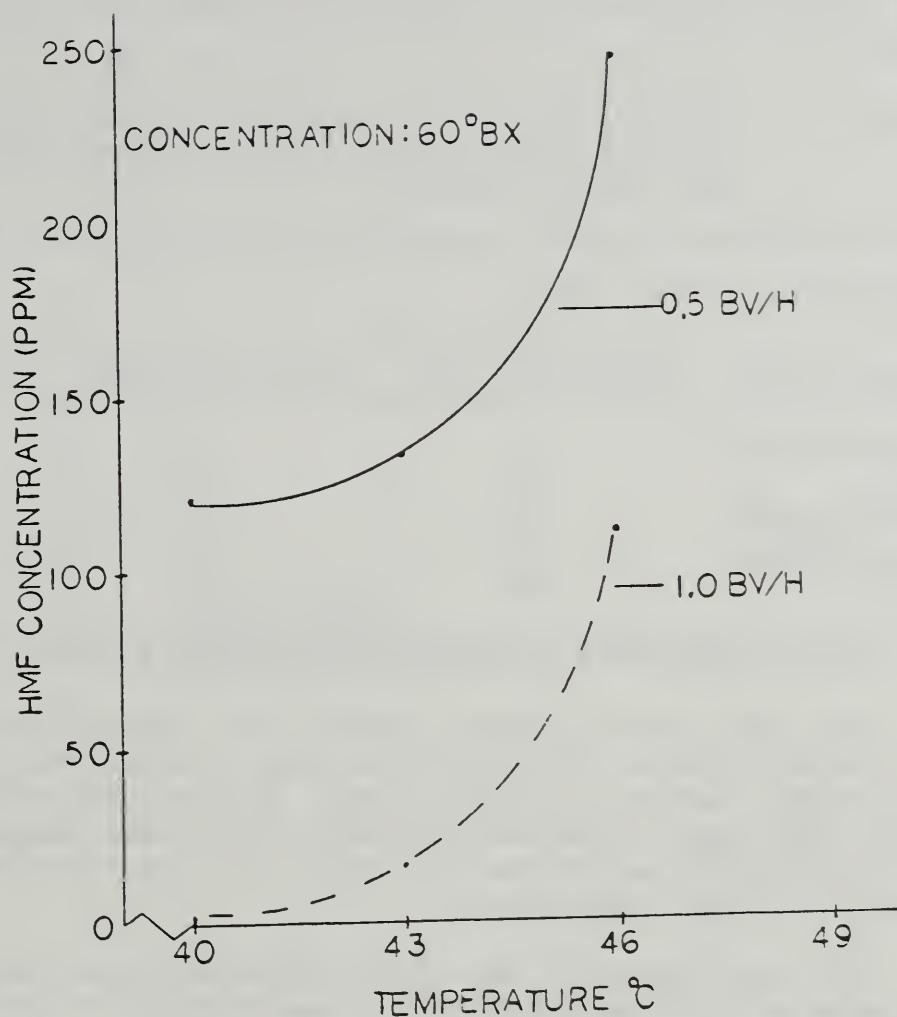


FIGURE 3.-- EFFECT OF SLIGHT CHANGES ON THE FORMATION OF HMF FOR THE MACRO-POROUS RESIN AT FLOW RATES OF 0.5 AND 1.0 BV/H.

For the sake of continuity, the reaction rate constants reported in this paper were determined according to the following expression used by Siegers and Martinola (1985):

$$\ln (1-X_S) = - K \cdot t$$

Where $1-X_S$ = unsplit sucrose
 t = space time minutes
 K = rate constant

Listed in Table 1 are the rate constants calculated for the macroporous and gel-type resins for the test conditions of 40°C, 60°Bx feed, and a specific flow rate of 1.0 bed-volume

per hour. Also listed in Table 1 are values for rate constants of resins with similar values for average bead size and % DVB cross-linking as reported by Siegers and Martinola (1985).

Table 1. - Comparison of rate constants for the resins used in this study versus those published previously for similar resins.

Conditions: 60°Bx, 40°C

Resin Type	Bead Size, mm	% Cross-Linking	K, Min. ⁻¹
Macroporous	.60	5.5	.027
Gel	.60	4.0	.042
*Macroporous	.55	15.0	.027
*Macroporous	.55	5.5	.047
*Gel	.55	4.0	.050

* - Results published by Siegers and Martinola (1985).

The value for K, listed in Table 1 for the gel resin evaluated in these tests, indicates that it performed similarly with those studied in previous work. However, the macroporous resin used seems to parallel a resin with a much higher degree of cross-linking which has been shown by Siegers and Martinola (1985) to impede inversion.

The lower degree of DVB cross-linking and bead size of the macroporous resin employed should have produced a much higher rate of inversion at the given conditions according to past data. However a similar situation has been reported previously by Gilliland et al. (1971), where superior rates of inversion were reported for a gel-type cation exchange resin than for a macroporous. This data can also be questioned to the extent that Gilliland et al. (1971) did not specify the % DVB cross-linking. Apparently there are more factors affecting the quality of the resin's performance that have to be taken into account.

Having encountered the above-mentioned difficulties with the macroporous resin, it was decided to abandon it for the remaining part of the study. All efforts were concentrated on the feasibility of the gel-type resin.

Figure 4 details the data gathered for HMF formation as a function of flow rate and space time at 40°C for the gel-resin. It was determined that a maximum of 0.75 bed-volume per hour specific flow rate could be employed and still meet the HMF specification of 75 ppm.

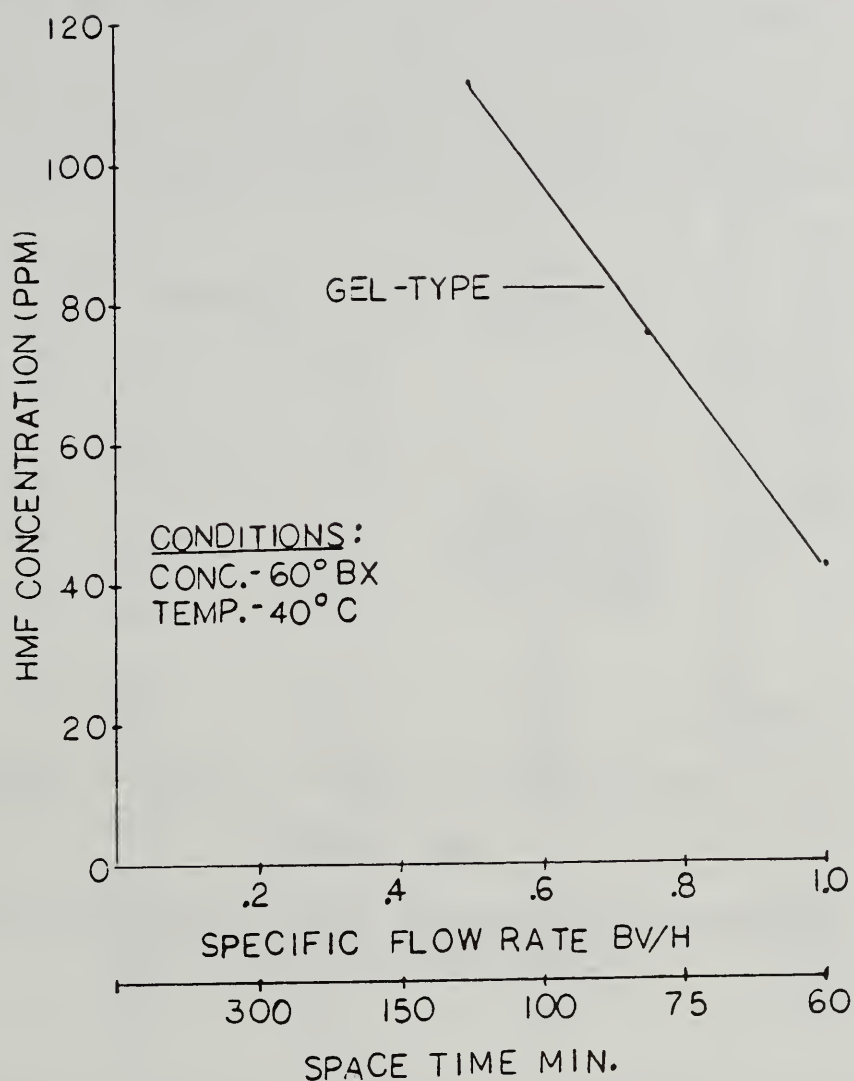


FIGURE 4.--HMF FORMATION VERSUS SPECIFIC FLOW AND SPACE TIME FOR THE GEL TYPE RESIN.

As a final experiment two columns containing the gel cation exchange resin were set up. One column was operated at 0.75 bed-volume per hour and the other at 0.5 bed-volume per hour as a means of comparison.

Figure 5 illustrates the two column's performance for percent inversion versus the amount of throughput in bed-volumes. A point of 90% invert was chosen as the shutdown point for both columns. Examining Figure 5 it can be seen that the 0.5 BV/H column began producing invert at a level of approximately 98.8% invert and ran for about 182 bed-volumes before hitting the 90.0% cutoff. The 0.75 bed-volume column started at approximately 96.0% inversion and ran for 113 bed-volumes.

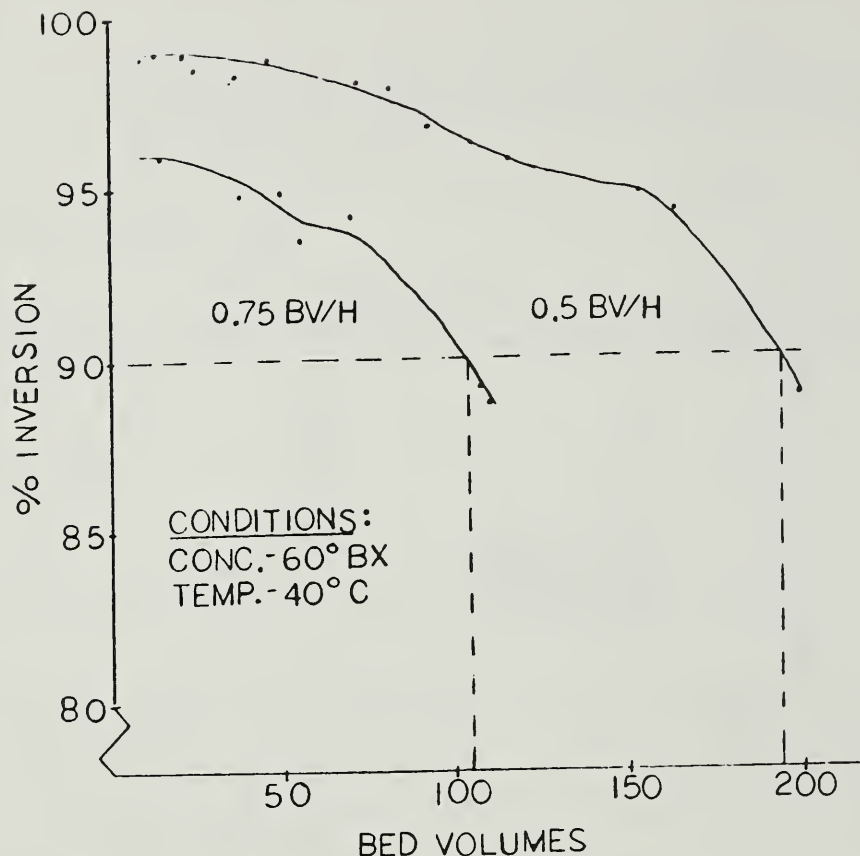


FIGURE 5.- PERFORMANCE OF GEL RESIN FOR SPECIFIC FLOW RATES OF 0.5 AND 0.75 BV/H

Listed in Tables 2 and 3 are the detailed analytical results for the operational cycles of the 0.5 and 0.75 bed-volumes per hour gel-type resin columns.

Examining the data, it can be observed that the ash and chloride concentrations, although trending slightly higher as the column cycle continued, exhibited no significant changes over the length of the entire cycle.

The color of the effluents also remained quite constant after showing an initial gain as compared to the feed syrup. This increase in color was unavoidably due to color body and HMF formation during the inversion process.

Tracing the production of the by-product HMF, a decrease in its formation as expected was seen as the efficiency or rate of inversion of the column declined with increasing throughput.

Table 2. - Detailed analytical results for the operation of the gel-type resin column at 0.5 bed-volume per hour, 40°C, and 60°Bx.

Avg. Feed Syrup Composition: % Ash 0.011, HMF 0.1 ppm, Color Index 15.6				
Bed- Volumes	*% Ash (Wet basis)	*Cl, ppm (Wet basis)	HMF, ppm (Wet basis)	Color Index (@ 420 nm, 50°Bx)
8	-	-	-	21.2
10	-	-	112	-
12	.021	-	-	22.1
14	-	-	114	-
19	.018	8.3	-	-
21	-	-	-	23.1
23	.019	-	-	-
28	-	-	-	24.0
32	-	-	-	23.6
33	.018	8.3	112	-
38	.021	10.5	-	-
42	.021	9.5	-	-
47	-	-	108	-
73	.023	8.2	107	-
83	.020	11.9	108	21.7
88	.024	8.3	106	-
95	-	-	-	22.6
100	.023	10.5	-	-
105	-	-	101	-
117	.023	13.1	99	24.5
156	.021	14.2	97	20.1
173	.026	17.5	95	26.0
185	.025	15.1	88	27.0
200	.026	18.9	76	-

* - The % ash was determined conductimetrically, and chlorides were analyzed by the Mohr method.

Table 3. - Detailed analytical results for the operation of the gel-type resin column at 0.75 bed-volume per hour, 40°C, and 60°Bx.

Avg. Feed Syrup Composition: % Ash 0.011, HMF 0.1 ppm, Color Index 15.6				
Bed- Volumes	*% Ash (Wet basis)	*Cl, ppm (Wet basis)	HMF, ppm (Wet basis)	Color Index (@ 420 nm, 50°Bx)
12	-	-	-	17.5
15	-	-	75	-
18	.021	-	-	-
20	-	-	77	18.8
29	.018	9.9	-	-
32	-	-	76	18.6
40	-	-	-	17.9
50	.018	8.4	74	20.2
63	.021	11.6	-	-
70	-	-	72	-
108	-	-	67.6	-
110	.022	10.5	-	-

* - The % ash was determined conductimetrically, and chlorides were analyzed by the Mohr method.

Accessing the total data generated for the two particular resins evaluated in this study, it became evident that our goal to produce a one-step premium medium invert syrup meeting certain predetermined specifications would be impossible using the macroporous cation exchange resin.

Additionally, although it has been shown that the product could be produced with the gel-type resin, this process would be highly unfeasible due to the narrow processing constraints that would have to be placed on it; for example, the critical controls on temperature and space time.

Another viable conclusion arising from this study was that, although the gel-type resin was expected to perform better than the macroporous due to the slightly lower degree of DVB cross-linking, it was not anticipated that the macroporous resin would perform as poorly as it did. In lieu of this and the other previously mentioned studies on these resins and such processes, it appears each resin's performance cannot be classified totally by its physical and chemical specifications alone but should be evaluated to determine its exact functionality in the application it will be utilized for.

SUMMARY

A laboratory evaluation comparing the inversion of a sucrose solution by strongly acidic cation exchange resins is described. Two different commercially available resins, one gel-type (average bead size 0.6 mm. and 4.0% DVB cross-linking) and one macroporous (average bead size 0.6 mm. and 5.5% DVB cross-linking), were chosen for comparison.

The purpose of the evaluation was to ascertain the feasibility of producing a premium grade total invert syrup in a one-step treatment of white granulated sugar syrup with the cation exchange resin.

Pertinent laboratory data in regard to the laboratory column's operating parameters % inversion, flow rates, and temperature as well as analytical results were presented. Special considerations were given to the formation of 5-hydroxymethylfurfural (HMF) during the inversion process.

Results of this study indicated that, in terms of the two resins evaluated in this work, the performance of the gel-type cation exchange resin proved far superior to that of the macroporous resin.

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DISCUSSION

J. Bruijn, Sugar Milling Research Institute - Why did you feel that the macroporous resin should perform better, and why is the gel-type actually performing better?

Wnukowski - This relates back to some information in Mr. Percival's paper. Literature values for macroporous resins of similar bead size and cross linking show inversion of 99% or better at a flow rate of one bed-volume or more per hour--of course, this was after prior demineralization of the liquor. We anticipated results like this, but apparently this is not the case for all resins.

Margaret A. Clarke, S.P.R.I. - What was the preparation method for the fine liquor used in input on the resin?

Wnukowski - That was our normal fine liquor, number one sucrose syrup in the refinery. I believe it was our granulated sucrose melted with no other treatment.

Clarke - Did you remelt white granulated?

Wnukowski - No, it was taken straight from our refinery.

Clarke - You have all sorts of refinery processes.

Wnukowski - It was a char-filtered liquor.

Clarke - The liquor was from press-filtration and bone char with no clarification?

Wnukowski - Exactly.

Andrew Ho, Redpath Sugars - Apparently you have some problem with HMF in your products. Have you considered other treatments to remove this?

Wnukowski - Mr. Percival suggested, in his paper this afternoon, that anion resins might remove HMF. We've also heard that activated carbon may remove HMF, but we haven't studied this. Our interest has been to use a single-pass treatment and meet our specifications.

David Jayes, Rohm and Haas Co. - Did you measure the percent conversion to the hydrogen form, following your pre-conditioning?

Wnukowski No, we didn't.

Jayes - The point is that two bed volumes of 2N HCl with a twenty minute contact time, twice, may not convert the macroporous resin as completely to the hydrogen form as compared to the gel resin.

J. Williams, Tate and Lyle Sugars - You have a very small amount of ash in your feed liquors; what was the pH of your product?

Wnukowski - The pH of the product off the column was 2.5-2.6.

Joseph F. Dowling, Refined Sugars, Inc. - We've used both gel and macroporous through the years. With macroporous, we always got higher inversion rates, but we were feeding a char liquor with a higher ash level than you quote.

Stanley E. Bichsel, American Crystal Sugar - Some years ago, we were working in this area. We noted that there seemed to be a relationship--all things being equal with concentration, temperature, bed volume--between the amount of ash and the rate of inversion. We were using melted white sugar as input. The higher the ash levels (of up to 0.01, conductivity, versus 0.004) seemed to decrease the inversion rate. Could you comment on that?

Wnukowski - We did not vary ash levels in our experiments, but confined ourselves to our normal refinery throughput, which is pretty uniform in ash content.

THE PRODUCTION OF INVERT SYRUPS USING IMMOBILIZED ENZYME

Michael J. Daniels

British Charcoals and Macdonalds

SUCROSE INVERSION

Invert syrups have been produced for many years, using various techniques to produce a range of proprietary products. The feedstocks are usually high in color and relatively low in purity, so, consequently, the products are not particularly pure, consistent or stable.

Some producers have considered this advantageous since it offers a means of marketing color, ash and in some cases the invert which was present in the feedstock. It is true that some companies produce high specification inverts for particular markets, but in general the syrups could not be considered to be of equivalent quality to the crystalline sucrose which the same company produces.

Invert sales are declining throughout the industry for a variety of reasons, cheaper alternatives, changing consumer requirements and in some cases a reluctance on the part of the refinery to continue production due to the practical problems which arise when invert is produced together with sucrose.

HFCS is, broadly speaking, an invert syrup which has displaced sucrose in many major markets due to price advantage, but it is not always appreciated that it is now the quality standard by which invert syrups should be judged. This means low color, good color stability, low ash (particularly chloride), low HMF and limits on sulphite and aldehyde content.

This is the type of invert I wish to discuss and, in particular, to demonstrate the advantages of immobilized invertase for the production of high purity syrup.

The traditional method of production, using acid treatment at elevated temperature and pressure, can be carried out in batch or continuous mode so as to minimize the residence time and temperature, but the chloride or phosphate content will be relatively high and the color and decomposition products produced

will significantly affect the quality. The process can be operated at high solids, but this makes any final blending to specification difficult.

The use of ion exchange for inversion does not produce the same ash contamination and the color and decomposition produced is usually less than with acid inversion, but nevertheless the purity is adversely affected.

The production of high invert syrups (95%+) is extremely difficult using either acid or ion-exchange resin and obtaining precise degrees of inversion at intermediate levels is sufficiently difficult that blending is often required to obtain the final product specification.

Liquid invertase is added to a tank of 50-60° Brix sucrose at approximately 50° C and the required degree of inversion would be obtained after some 10-30 hours residence. The conditions are relatively mild due to the low temperature but the long residence time leads to contamination and hence the production of some color and impurities, particularly since there is protein present in the form of enzyme.

The relative costs of these three processes are difficult to calculate due to the large variations in factory equipment and procedures which are employed but one can make general comparisons.

<u>Acid Inversion</u>	<u>Ion Exchange</u>	<u>Liquid Invertase</u>
Low material cost	Medium material cost	High material cost
High energy cost	Medium energy cost	Medium energy cost
High capital	High capital	Medium capital
No evaporation cost	Evaporation cost	Evaporation cost
Minimum effluent	High effluent	Minimum effluent
High ash content	Low ash content	Low ash content
High color	Medium color	Medium color
Poor stability	Medium stability	Medium stability
Poor inv. control	Poor inv. control	Good inv. control
Low max inversion	Low max inversion	High max inversion
Low contamination	Low contamination	Medium contamination

IMMOBILIZED INVERTASE

Immobilized invertase is a granular product which is operated in a column, the syrup is pumped downwards through the bed and the inversion occurs in a single pass so the production is continuous.

An immobilized enzyme system utilizes a very high enzyme concentration such that inversion can be performed in minutes or seconds of residence time and there is virtually no color or impurity production.

The production plant is flexible and the productivity is high so it follows that the capital cost is relatively small and the installation costs would be negligible.

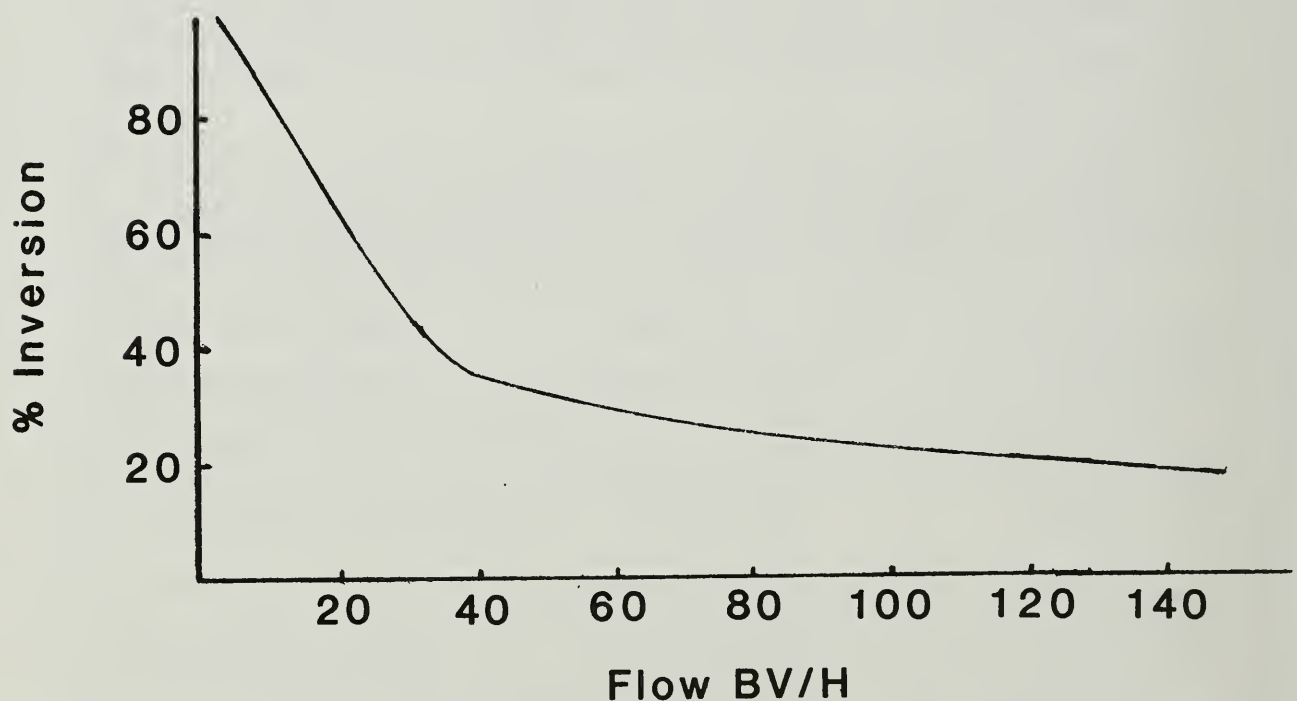


Figure 1.--Performance of immobilized invertase on 50° Brix sucrose.

Figure 1 shows the action of immobilized invertase on 50° Brix sucrose, the x-axis is bed volumes/hour which is a convenient and scaleable measure of flow rate and the y-axis represents percentage inversion defined at 100 - % remaining sucrose.

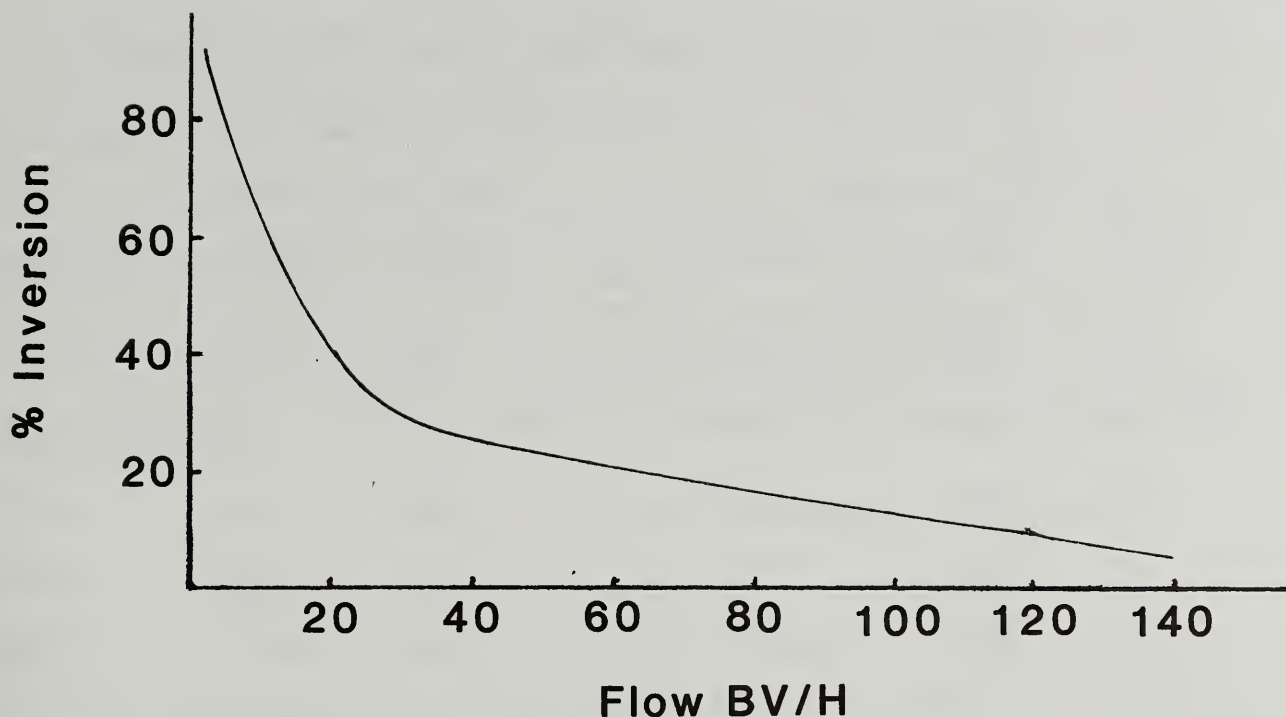


Figure 2.--Performance of immobilized invertase on 60° Brix sucrose.

Figure 2 shows the performance using 60° Brix sucrose and it can be seen that the performance decreases markedly at this increased concentration due to the reduced concentration of the water required to perform the hydrolysis.

The degree of inversion can be controlled very precisely by variation of the flowrate and if in-line polarimetry is employed, the product specification can be set and controlled, compensating for any change in input solids or invert content.

Table 1.--Typical enzyme performance per liter enzyme

50° Brix Sucrose			60° Brix Sucrose		
% Inv	Flow l/h	DS Kg/h	% Inv	Flow l/h	DS Kg/h
100	4.0	2.46	100	1.3	1.00
95	8.0	4.92	95	3.0	2.30
50	30.0	18.45	50	16.5	12.77
25	94.0	57.81	25	51.7	40.02
15	180.0	110.70	15	100.0	77.40

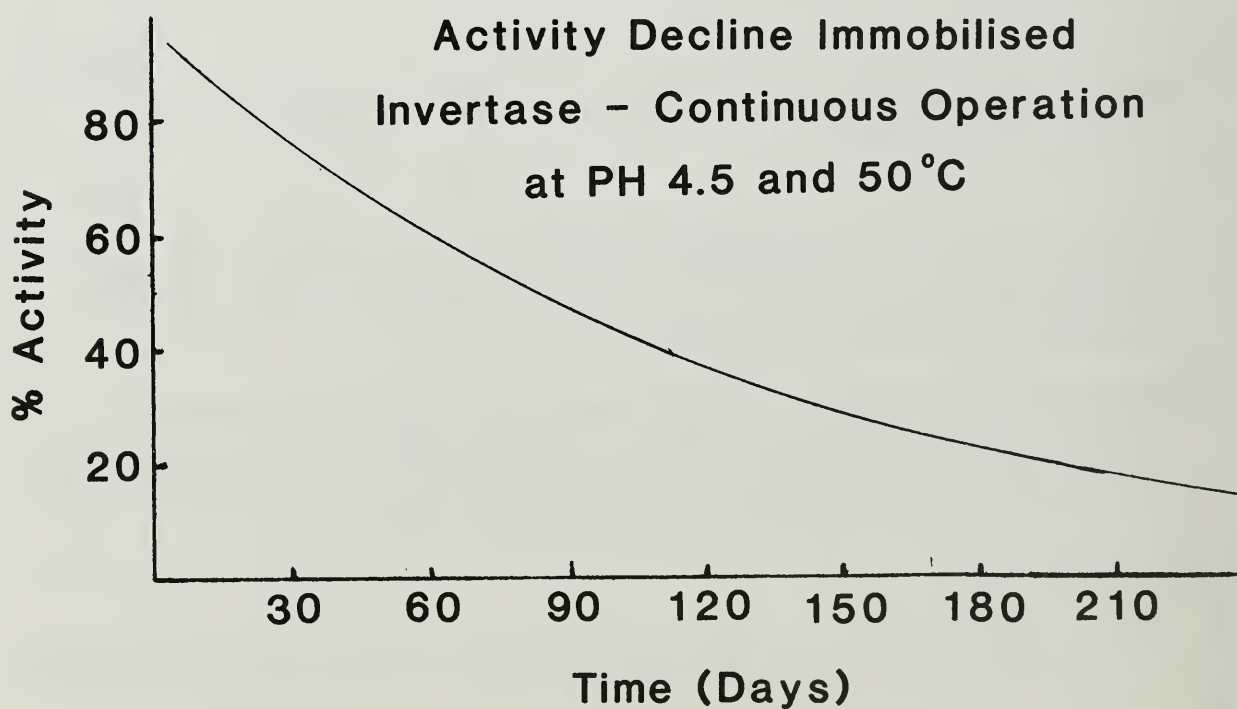


Figure 3.--Enzyme decay of immobilized enzyme under continuous operation.

The activity of immobilized invertase decays as it is used and with remelt sucrose one could expect to have 50% of the initial activity

after some 90 days of use. This type of decay continues during two more "halflives" so that, after 270 days factory lifetime, the activity has decayed to 12.5% of the initial and the enzyme would normally be discarded at this stage.

In practice, the usual procedure is to add fresh enzyme to the column at regular intervals to maintain productivity; this is more efficient in terms of enzyme usage.

During three halflives, the average productivity would be very close to 50% of the Day 1 productivity and so it is relatively easy to calculate the total production obtained per liter of immobilized invertase.

Defining B as the Day 1 flow to produce the required result and H as the halflife.

$$\text{Total Vol} = B/2 \times 24 \times 3 \times H$$

if the halflife is 90 days then:-

$$\text{Total Vol} = 3270 \times B$$

At 50° Brix this means $1.23 \times .5 \times 3240 = 1992.6 \times B$ Kg and

At 60° Brix this means $1.29 \times .6 \times 3240 = 2507.8 \times B$ Kg.

Thus the information provided in Table 1 can be used to produce the productivities per liter of enzyme for a range of degrees of inversion.

Table 2.--Typical enzyme performance per liter enzyme

50° Brix Sucrose			60° Brix Sucrose		
% Inv	Total Vol	Total Sols	% Inv	Total Vol	Total Sols
100	12960 L	7970 kg	100	4212 L	3245 kg
95	25920 L	15941 kg	95	9720 L	7442 kg
50	97200 L	59778 kg	50	53460 L	41379 kg
25	304560 L	187304 kg	25	167508 L	129653 kg
15	583200 L	358668 kg	15	324000 L	250780 kg

Using a cost of \$40/liter for the enzyme, it is now possible to calculate the operating costs for the production of the types of invert considered.

These are costs based on using pure sucrose, the halflife obtained is reduced with more colored feedstocks and these costs could be doubled if very dark syrups are treated.

Table 3.--Typical enzyme performance per liter enzyme

50° Brix Sucrose			60° Brix Sucrose	
% Inv	Cost/100 gal	Cost/100 lb ds	Cost/100 gal	Cost/100 lb d
100	116.9 c	22.8 c	359.8 c	55.9 c
95	58.5 c	11.4 c	155.9 c	24.5 c
50	15.6 c	3.0 c	28.3 c	4.4 c
25	5.0 c	1.0 c	9.1 c	1.4 c
15	2.6 c	0.5 c	4.7 c	0.7 c

These costs can be loaded by 10% to allow for running and capital overheads, but it can be seen that the operating costs for immobilized invertase are low for intermediate degrees of inversion and although they increase significantly for syrups which are inverted more than 90%, this will be equally true for the alternative production methods.

In practice, columns can be operated at fairly high flow rates with syrups up to 60° Brix and at low flow rates the syrup concentration can be higher. There is no practical reason to limit the operating syrup concentration but there are economic ones.

Invert syrups are marketed at 70-75° Brix and although some concentration can be obtained by flash cooling and there is some concentration increase due to hydrolysis gain, there will be a need for evaporation when using syrups of less than 70° Brix concentration.

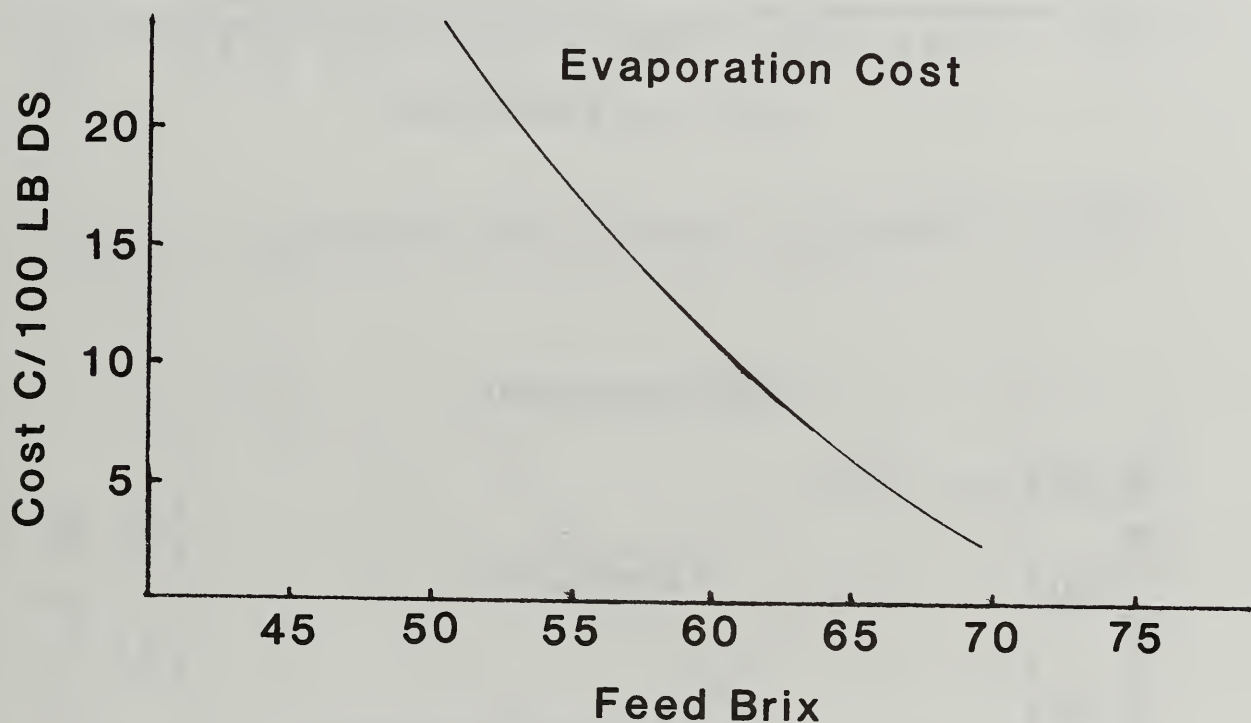


Figure 4.--Cost of evaporating invert syrup.

Figure 4 shows the cost of evaporating invert syrups which have been produced at a range of concentrations to a final concentration of 75° Brix. Combining these costs with the cost of inversion, which increases with increasing solids concentration, will provide the optimum solids concentration to operate each type of invert syrup.

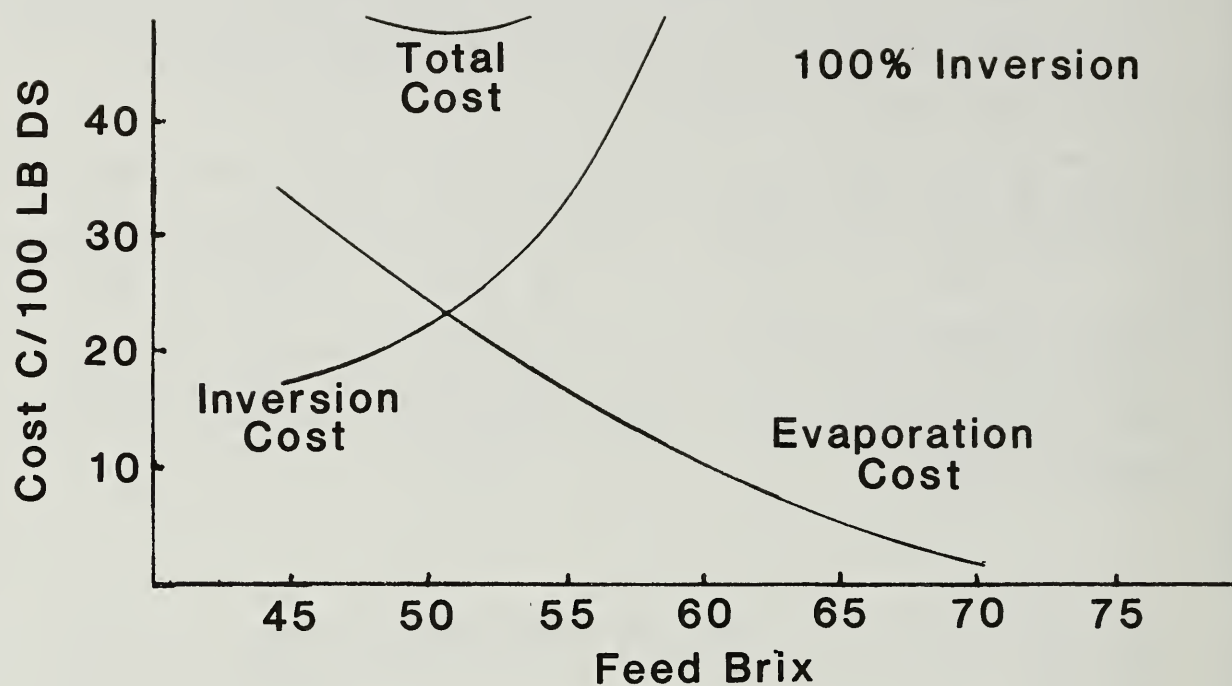


Figure 5.--Comparison of costs for 100% inversion.

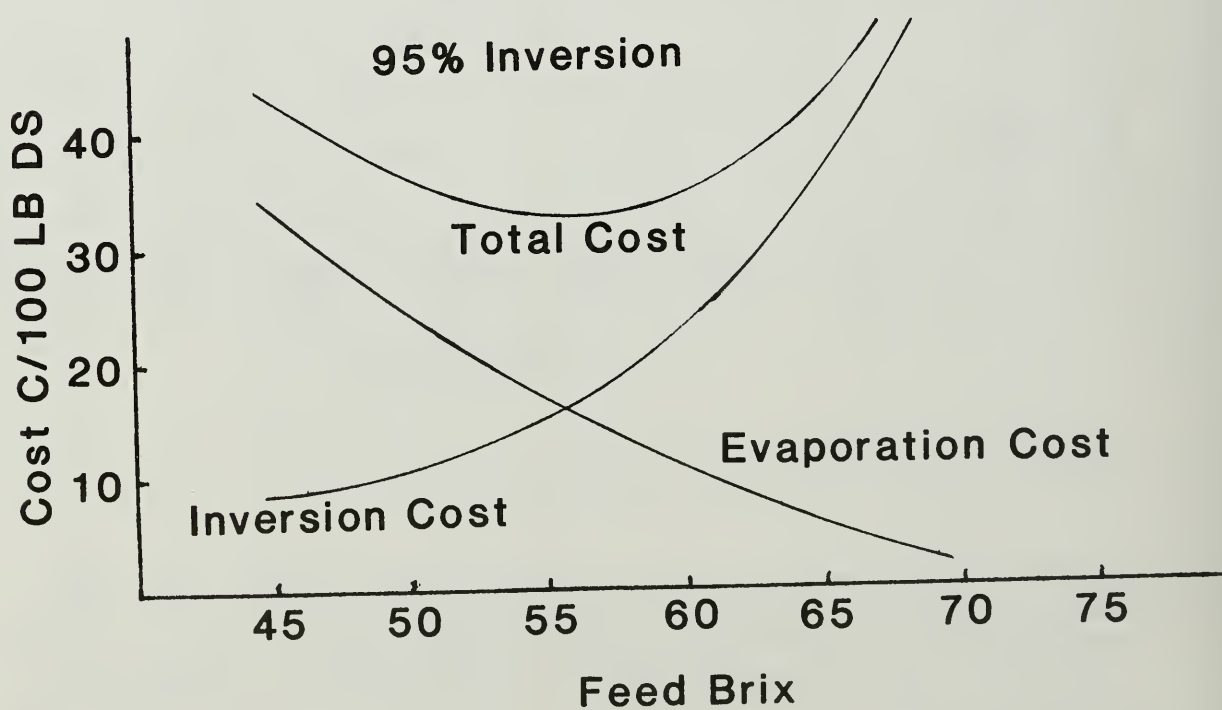


Figure 6.--Comparison of costs for 95% inversion.

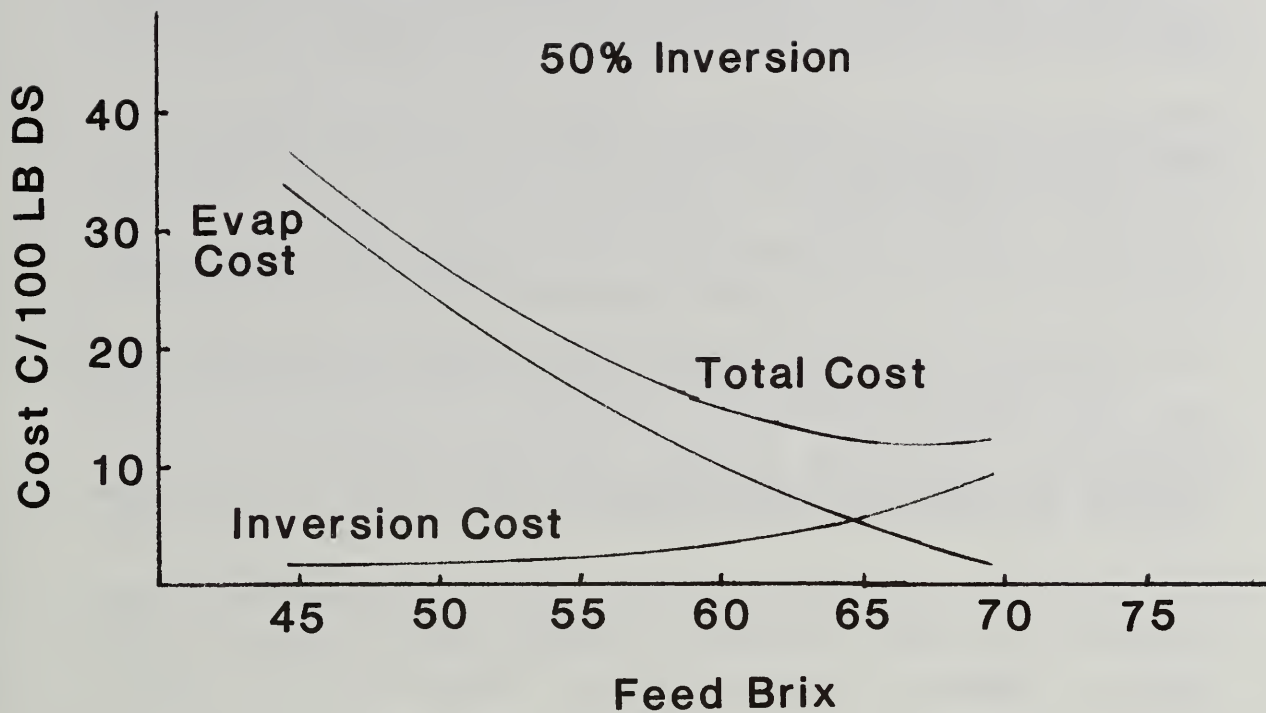


Figure 7.--Comparison of costs for 50% inversion.

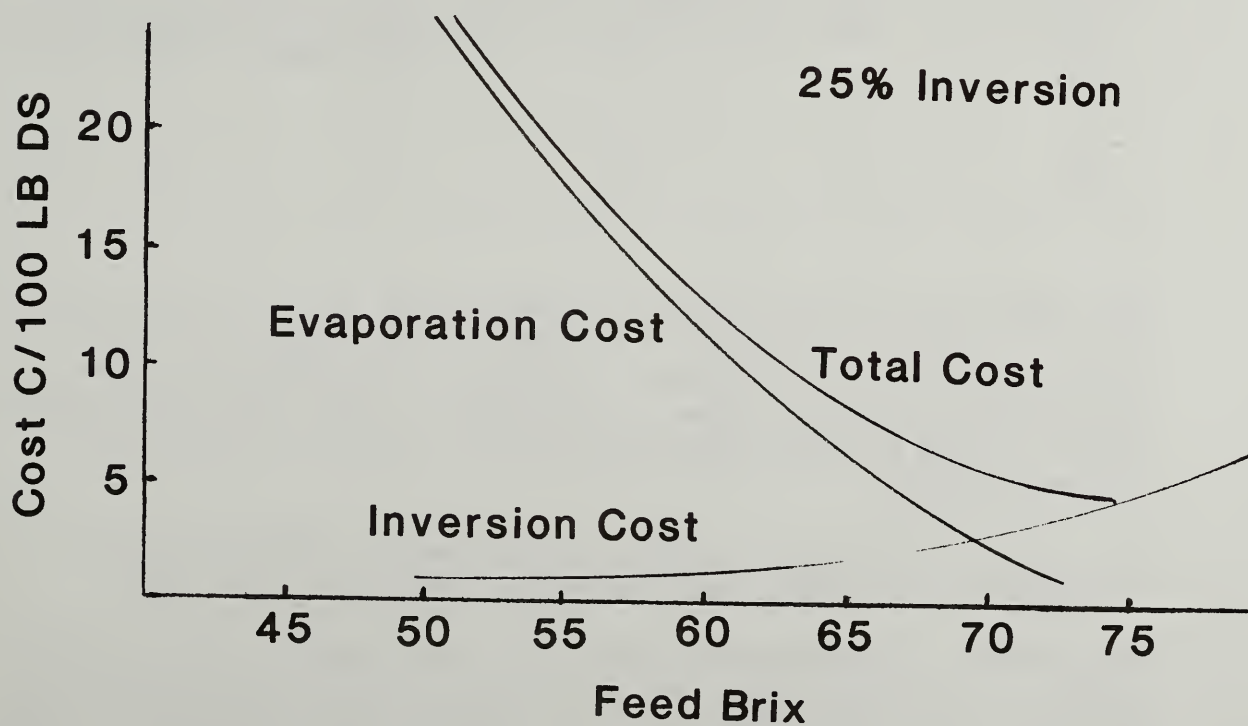


Figure 8.--Comparison of costs for 25% inversion.

The optimum Brix for complete inversion is around 50° Brix since the inversion cost is relatively high compared to the evaporation cost.

The optimum Brix has increased to around 55° Brix for 95% inversion, since the cost of inversion and the cost of evaporation are roughly comparable.

The optimum Brix for 50% inversion is around 67° Brix since the evaporation cost is the highest component.

The optimum Brix for 25% inversion (and below) is around 75° Brix, i.e. the finished syrup Brix. Although it may not be practicable to operate at this Brix, it means operation at a syrup concentration where only a finishing flash concentration is required to produce the final syrup.

Table 4.--Summary of inversion costs. (Costs are c/100 lb ds)

Inversion	Optimum Brix	Total Cost	Enzyme Cost	50° Bx Enz Cost
100	51	54	27	23
95	56	32	16	11
50	67	12	6	3
25	75	4	2	1

The evaporative costs can only be considered as part of the total costs if they are incurred as a result of the installation of an immobilized invertase system. If syrup is available at lower concentrations and the final evaporation is not a new cost element, then it would be more cost-effective to invert at lower syrup concentrations.

Thus, the last column in Table 4 provides the comparative cost information for immobilized invertase operated at 50° Brix.

The process stage costs of inversion using immobilized invertase appear to be competitive with existing processes, particularly if the simplicity and flexibility of operation are considered. Check filtration is all that is required after the column and this can remove a major source of contamination which occurs if sucrose and invert are processed through the same filtration system.

In the USA, HFCS has a significant cost advantage over sucrose which may or may not endure, but even a legislative change which altered this situation would not necessarily mean that customers would revert to crystalline sucrose since they are now effectively geared to using 95% invert syrup.

The fact that HFCS is only 95% monosaccharides has not really been exploited, but a high quality invert syrup should be superior, particularly where non-monosaccharides represent both a loss of product and additional purification costs, for example in sorbitol and mannitol production.

The purpose of inversion is to provide increased solubility, increased sweetness or glucose and fructose in the place of sucrose, and it is not always clear which function the customer is purchasing. In some cases, the inherent purity of a sucrose-based syrup would be better demonstrated by good color and low trace impurities.

Immobilized enzymes are still "new technology" but, in fact, their use is probably no more complex or technical than an ion-exchange system.

Their operating costs are comparable to traditional processes, when the obvious economics of existing methods are considered, but there are often substantial savings available due to reduced process costs in other areas.

In the case of immobilized invertase, there is the added advantage that the product from a column is superior to many of the syrups currently produced which could help to keep existing markets or even open new possibilities.

DISCUSSION

T. Chadwick, Holly Sugar Corp. - Are there any classes of impurities, other sugars for example, that come in the feedstock that can accelerate the deactivation of the immobilized enzyme?

Daniels - No; carbohydrates and ash have virtually no effect in their normal ranges of concentration. There is a problem with the organic materials associated with color: Certainly the more pure the syrup, in terms of color, going on to the enzyme, the longer will be the lifetime of the enzyme. The lifetime you'd expect with any pure syrups might be reduced by 25% or 50% perhaps with very dark syrups. The enzyme will not be killed immediately.

Enrique R. Arias, Sugar Cane Growers Cooperative of Florida - Your work has been very thorough, as shown by all your detailed graphs and tables. You said that the costs are comparative costs. Is it cheaper to produce invert syrups using immobilized invertase than other systems?

Daniels - The costs for immobilized enzymes are shown in Table 3. If you compare costs in the corn syrup industry, the cost of an enzymatic process--even to an unusual 100% conversion--is some \$12 per ton. That compared very well to the cost of saccharification using glucoamylase, for example. Comparison costs are a moving target. One producer may have no capital costs on his current installation because it's so old. Others may have no need for tight specifications--50% may be anywhere between 40% and 60% for their customers.

The simplicity of operation of immobilized enzymes is a major factor in operating cost calculations. If you consider, say, half the advantages of immobilized enzyme systems, then costs are comparable. If you consider all the advantages, including the fact that you can make some products you can't make any other way, then the enzyme costs are cheaper. It's a difficult comparison--enzyme system costs show up front, and other system costs may be hidden.

C. T. Dickert, Rohm and Haas Co. - On one of your slides (Figure 3) you showed a 50% reduction in activity after 90 days, and a further reduction in activity as time went on. You mentioned that you can add more enzyme periodically to compensate. Does the inactivated enzyme disappear? Does material have to be removed from the column if the enzyme disappears? You also showed a slide (Figure 2) that indicates a rather sharp drop in percent inversion as a function of bed volume per hour (flow rate). If the enzyme is disappearing, must an adjustment be made in the flow rate?

Daniels - First, let me be absolutely clear as to how we define the half-life: If there is a specific result with, say, 8 bed-volumes on Day 1, then half-life is the time after which you will achieve the same result with 16 bed-volumes. We're talking about reduction of flow rate in order to maintain product specification. Because of this change, and because the productivities are relatively high, it is quite reasonable to start with a half-full column. Deterioration of production is such--even if you are working to a

tight specification--that you can readily add whatever small percent of additional enzyme is required, and thus extend your operating life from 6 months to 9 months or 1 year before you need to think about decreasing flow rates. You keep topping up the half-full column.

R. Dickey, Refined Sugars, Inc. - You mentioned running inversion rates up to 25% and monitoring with HPLC analyses. Did you do HPLC at the 25% level, and if so, what kind of glucose-fructose ratios did you find?

We ran some laboratory-size columns of enzymes, and found a higher level of glucose than fructose at the 25% level.

Daniels - The action of all invertases, liquid or immobilized, form a range of intermediate trisaccharides and a few tetrasaccharides, which tend to be richer in fructose than glucose (e.g. fructose-fructose-glucose). Perhaps as much as 5% of the fructose is tied up in these oligosaccharides. If you continue to invert, these are broken down again to fructose and glucose, but in the intermediate stages, there is a shortfall of fructose.

With regard to oligosaccharides in corn syrup, there is 5% material there that is not monosaccharide--but in that case it is permanently not monosaccharide. It's isomaltose, equilibrium maltose, and intermediate oligosaccharides that can't be broken down. This material is not fermentable; it's not sweet; it's just there for the ride.

The type of oligosaccharides in the 25% case are an intermediate product; they have sweetness and will break down with further treatment.

Mary An Godshall, Sugar Processing Research, Inc. - Would you comment further on the production of sorbitol and mannitol. Are these also made from sucrose?

Daniels - Once again this comes back to the oligosaccharides. We must remember that high-fructose corn syrup is only 95% monosaccharides. To make sorbitol or mannitol, further purification is necessary to remove that 5% oligosaccharides. If sucrose is inverted to make a starting material for sorbitol or mannitol, it's not necessary to invert 100% because usually the hydrogenation process (to make the alcohols) will invert the last 4 or 5% of sucrose. Using inverted sucrose, sufficient money can be saved downstream, because the removal of oligosaccharides is not necessary; that is, it is actually cost effective to make sorbitol and mannitol from inverted sucrose rather than HFCS. The cost margin between HFCS and sucrose is not enough to outweigh the downstream processing.

A. M. Bartolo, Imperial Sugar - Could you tell me what are the components of your evaporation cost? If one component is a fuel cost, what is the basis for that fuel cost?

Daniels - Evaporation cost consists of a fuel cost and a small capital cost, and is based on U.S. costs, not U.K. costs. There are many factors that affect evaporation costs--number of effects, vapor recompression, etc.--so the figures in this paper are only a general guide, using average U.S. costs.

Margaret A. Clarke, S.P.R.I. - Would you comment on the possibility of using a fixed dextranase enzyme in sugar production, particularly with respect to the raw sugar factory?

Daniels - Fixed dextranase is relatively easy to produce; however, I am not convinced that the industry actually needs it. When a sugar factory has a dextran problem, they'll pay a lot to get rid of it for the short time it's a problem. But when the problem is not there, the factory does not want all the capital tied up and has no requirement for that treatment.

A fixed dextranase system would be relatively easy to establish, but we feel that there is little reason to divert resources from products that people want to buy to a system that the industry may need but may not be prepared to pay for.

PRODUCT APPLICATION RESEARCH - A TOOL TO SUPPORT MARKETING

Juha Ahvenainen and Juhani O. Kuusisto

Finnish Sugar Company Ltd.

INTRODUCTION

Sugar is a product whose marketing situation has traditionally been very stable until the last one or two decades. The main problem in sugar marketing is the saturated market; people cannot eat much more sugar and sugar-containing products. During the 70's there was a wide antisugar nutritional campaign, which weakened the image of sugar and considerably decreased sugar consumption. In addition, new competing sweeteners have taken place in the traditional sugar market. For instance, starch-based syrups have occupied a great deal of the industrial market for sugar, especially in the U.S.A. There are also new, continually developing intensive sweeteners which are becoming more popular all over the world. Aspartame in the soft drink industry is a good example.

It is obvious that something has to be done to protect the markets for sugar, to maintain the current situation or to improve it if possible. In this contribution we would like to give an example of the strategy which has been adopted in the Finnish Sugar Company. The strategy mainly consists of a sugar image marketing campaign and product application research and service.

IMAGE CAMPAIGN OF SUGAR

The brand image of sugar has been very negative in Finland since the 70's. At that time irrelevant discussions about sugar started, and sugar seemed to be "white poison" in a nutritional and healthy point of view. These negative attitudes spread easily

among individual consumers and in the food industry. This campaign against sugar led to the situation where the sales dropped over 30 percent.

As a consequence of faulty information, sugar was considered an industrial and artificial product that contains additives and has no good nutritional benefits. There was a very deep discrepancy between the actual product and illusions. So we decided to start a campaign for sugar.

The basic ideas of the sugar campaign

The main claims of our campaign are:

- A) Sugar is a natural product.
- B) Sugar has a central role at the birth of life on the earth and in the cycling of nutrients.
- C) White sugar is separated from plants by the sugar industry and it is exactly the same sugar as in the plants.

We did not want to start any yes/no debate about sugar but to give the right information about it and its benefits to different kinds of people and customers. Therefore, we chose the multimedia solution for four different target groups. These groups are consumers, industrial customers, catering people and experts ("opinion leaders").

The basis of the campaign is the new sugar symbol for which we had a public designing competition in the spring of 1984. With this symbol we indicate that sugar was born in nature where the plants convert the energy of the sun to sugar.

The media we use are symbol, TV advertising, magazines, daily papers and large roadside posters, packings and two new information magazines coming out four times a year:

- Sugar News for industrial customers
- Sugar in Life for influential persons in nutrition and food culture

The results of the campaign have been very promising after the work of 2-1/2 years. At the beginning of the campaign the naturality of sugar was considered equal to margarine. After a few months' campaign the naturality was raised to the same level as wheat flour and is approaching honey.

PRODUCT APPLICATION RESEARCH

The sugar marketing campaign is based on scientifically proved facts. In this field the product application research and service can best assist marketing. The aim of the research is to advise the customers to choose the most suitable product and product quality to meet their needs. In the background there is, of course, the point of increasing the sales or at least maintaining the current situation. Product development can also improve profit by increasing the processing state of the products.

The activities of the product application research department can be listed as follows:

A) Literature studies

- following the international development in foodstuff and sweetener field
- product information for customers
- information for marketing and research staff in our own company

B) Product development

- new products
- products replacing imports and supporting exports
- new blends of existing products

C) Research of product properties

- physical and chemical properties
- testing in food applications

Sugars, starch products, sucrochemical products, intensive sweeteners

D) Product applications cooperating with customers

- confectionery
- jams, juices, vegetable and fruit conserves
- beer and soft drinks
- bakery products
- wines and liqueurs

The assortment of Finnsugar sweeteners includes both cane and beet based sucrose products, starch hydrolysates and sucrochemical products such as fructose, xylitol and sorbitol. Sucrose products are the largest and economically the most important group of the assortment.

Product application research is closely related to marketing, and the operative staff consists of marketing people and specialists in the main customary areas such as confectionery, beverages and bakery products. The laboratory is equipped with a confectionery pilot plant and with analytical instruments needed in this work. More analytical service is ordered from the research centre of the Finnish Sugar Company or from outside the company if needed. The production trials usually take place at the cooperating customer's plant.

CASE STUDY

The comparison of different sweeteners in pectin jellies is given as an example of product application research. The aim of this kind of study is not to give the final recipes to the customer but to increase the knowledge and to help customers develop their own products.

Material and methods

The sweeteners compared in the study were fructose, glucose, sorbitol, xylitol produced by Xyrofin Ltd., U.K., and crystal sugar produced by the Finnish Sugar Company, Finland. Other raw materials which were equal in each test were pectin (Genu HM-Pectin 0 150° USA-SAG Slow set by Copenhagen Pectin Factory Ltd., Denmark), sodium citrate (trisodium citrate by Storge Chemicals, U.K.) and citric acid (citric acid monohydrate by Pfizer Chemicals, Ireland). Hydrogenated glucose syrup, Lycasin (Lycasin 80/55, Roquette Freres, France), was used as a doctoring agent for preventing the crystallisation of sweeteners.

The formula for pectin jelly was developed by trials with different ratios of xylitol and Lycasin. The texture of samples was tested by the Lloyd texturometer (T5K by J.J. Lloyd Instruments Ltd., U.K.). The samples were stored one month in climate chambers with different relative humidity conditions (RH 75% and RH 50%). The best recipe alternative of these preliminary tests was taken as the basic formula.

Test jellies were made on a small scale process, 1 kg per batch. All tests were made with the same basic formula, only the sweetener was changed.

The basic formula for pectin jellies

A)	Water	30.0	liter
	Sodium citrate	0.4	kg
	Citric acid	0.37	kg
B)	Pectin	1.5	kg
	Sweetener	5.0	kg
C)	Sweetener	30.0	kg
	Lycasin	55.0	kg
D)	Citric acid, 50% solution	0.74	kg

Process

- 1) Water (A) was measured into a boiling kettle. Citric acid (A) and sodium citrate were added and dissolved.
- 2) Dry pectin was blended with the sweetener (B), and this blend was added to the water while stirring.
- 3) The solution was heated slowly with stirring until it started to boil.
- 4) The sweetener was added avoiding cooling of the batch and dissolved by boiling. Lycasin was added.
- 5) The batch was boiled up to 110°C (230°F).
- 6) Citric acid solution (D) was added and the jelly was deposited immediately in starch.

After depositing pectin jellies they remained on starch trays for 24 hours at room temperature. After demoulding jellies the gel strength was measured with a texturometer. Water activity was measured by a hygroskop (Rotronic Ag., Switzerland) and water content with a Karl Fisher titrator (Mettler, Switzerland).

The shelf line quality properties were tested in a climate chamber with two different relative humidity values (52% and 75% RH). Water absorption and desorption were measured by weighing. Constant humidity conditions were made by saturated salt solutions, RH 52% with magnesium nitrate and RH 75% with sodium chloride.

Results and discussion

Table 1. Properties of the jellies made with different sweeteners.

Jelly made with	pH	$^{\circ}\text{Bx}$	DS	a_w
Fructose	3.6	79.9	80.6	0.66
Glucose	3.6	80.4	81.2	0.65
Sorbitol	3.6	79.1	80.7	0.65
Sucrose	3.6	80.6	81.0	0.67
Xylitol	3.6	78.1	81.2	0.63

Water activity values (a_w -values) of the jellies were lower than in most traditional jellies. This is due to Lycasin which gives a lower a_w -value than standard jelly containing sugar and glucose syrup.

Gel strength (Fig. 1)

This formula was specially made for xylitol jelly and all other sweeteners tested that gave stronger gels. We have noticed that with xylitol approximately 4% higher dry substance is needed compared to sucrose in order to get the same gel strength. Glucose and fructose formulations gave nearly the same kind of gels and gel strength which is about 2/3 of sucrose gel strength. It is well known that using invert sugar with sucrose a softer gel is achieved than with sucrose alone. It is possible also to improve the shelf life of jelly by using invert sugar.

Water absorption (Figs. 2 and 3)

All test jellies were hygroscopic and water absorption happened very fast at RH 75%. Xylitol and sorbitol jellies absorbed water more quickly than the others. This phenomenon is understandable because all alternative sweeteners tested were hygroscopic including also Lycasin. Equilibrium relative humidity (ERH) values were also low compared with storage conditions. At RH 52% no remarkable changes were noticed. All sweeteners tested had good humectant properties in dryer conditions and that is why changes happened slowly.

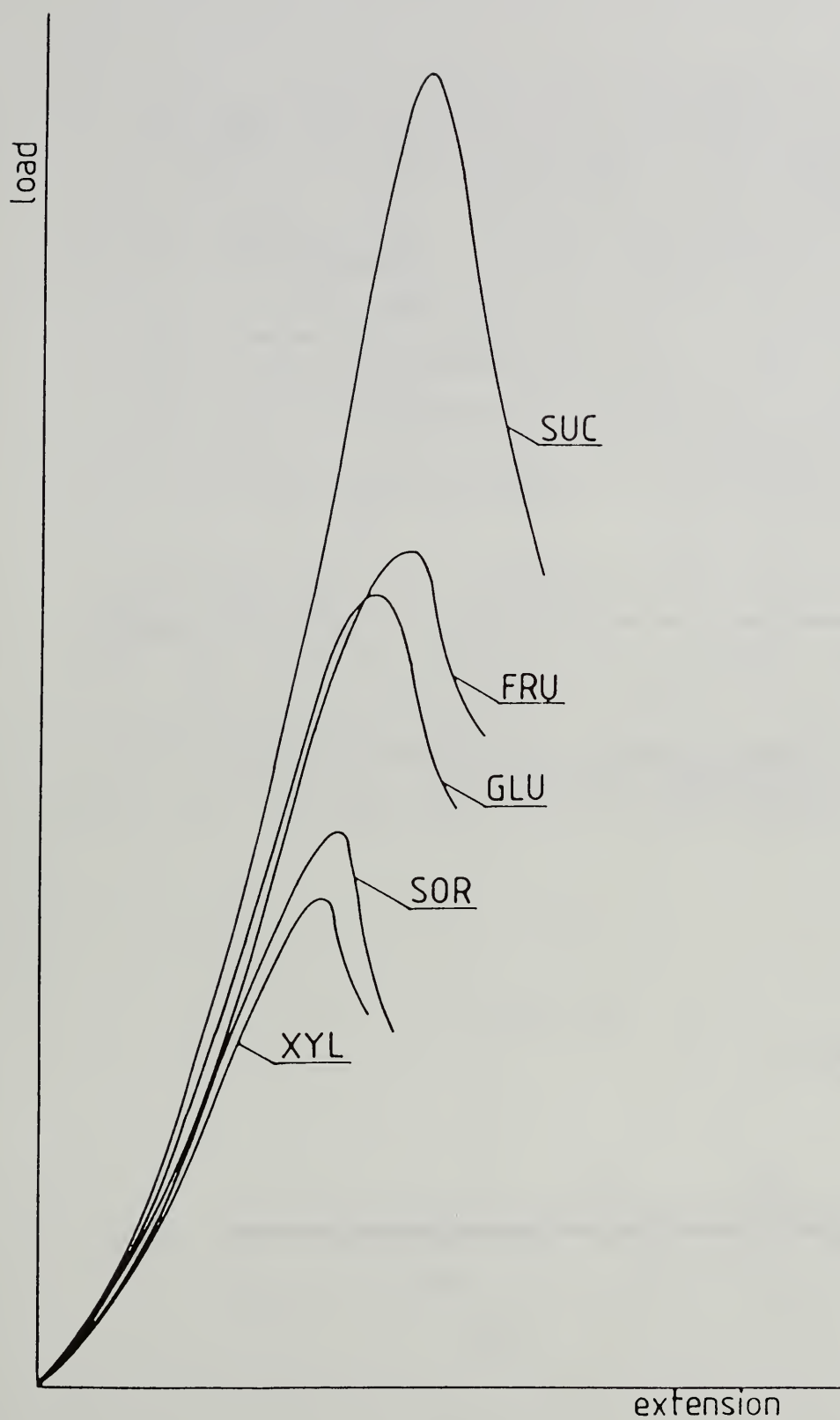


Fig. 1. Gel strength of the jellies made with different sweeteners.

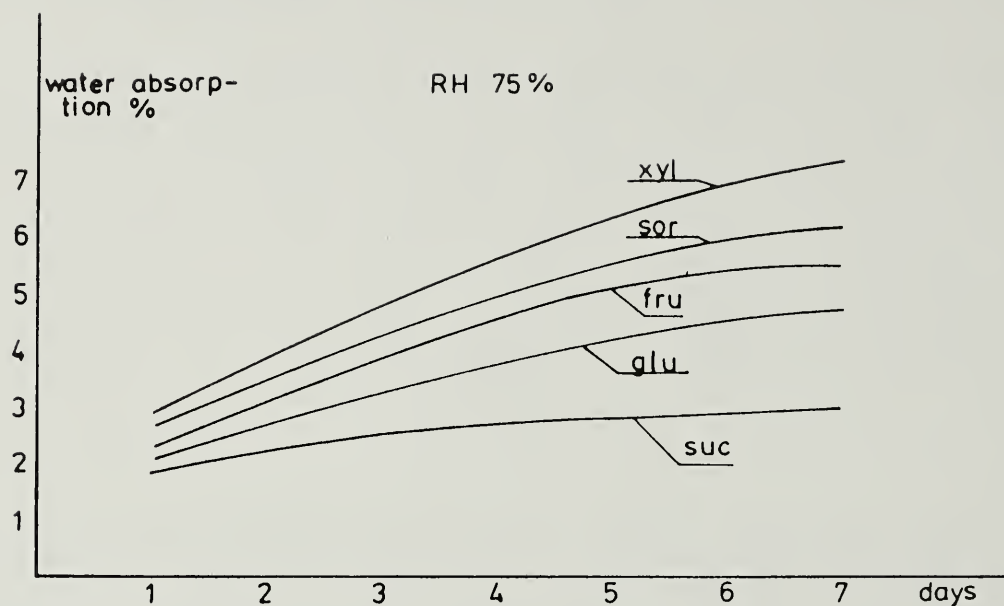


Fig. 2. Water absorption of the jellies at 75% relative humidity.

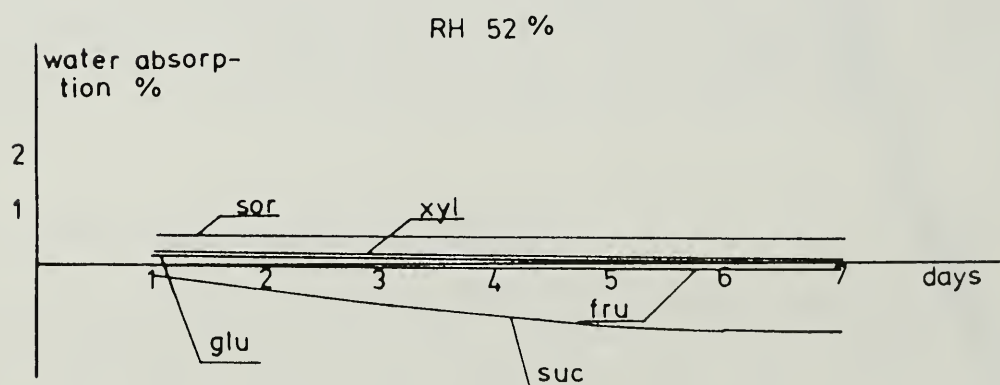


Fig. 3. Water absorption of the jellies at 52% relative humidity.

CONCLUSIONS

According to the results of this study it is possible to make good pectin jellies by using alternative carbohydrate sweeteners. In this case the aim of the work was to give knowledge for developing diet products where carbohydrates are other than sucrose and glucose. In the final product development work it is important to concentrate on dry substance and pectin concentration because the gel strength is much lower with other sweeteners than sucrose. The products are also hygroscopic which has to be taken into account when packages are planned. By using the traditional recipe including sucrose the tolerance and variation in the formulation are much wider than with alternative sweeteners.

By making this kind of application tests we can compare the specific properties of sweeteners in the same products and at the same conditions. We will not optimize formulas for customers but only give them basic knowledge and some ideas what kind of properties our sweeteners give in their products. Confectionery manufacturers can use our information and the result of application research as a basic material for their own product development.

DISCUSSION

T. Carreja, Sugar Cane Growers Cooperative of Florida - My question is simple: what are the costs of this kind of product application research?

Ahvenainen - In our case, the annual cost is about \$200,000 to \$300,000 per year, on an annual turnover of \$200 million. We are a small country, so the lab is quite small. There are capital costs when you first set up the lab and equipment, depending on how much you have already. A very high estimate for that is \$500,000.

N. W. Broughton, British Sugar plc - In Finland is there any kind of centralized research institute that might carry out this kind of work for the sugar industry on a non-commercial basis, perhaps on a contract research basis? For example, if someone wants to know the difference among various sweeteners for making pectin jellies, is there a central organization they can ask?

Ahvenainen - In principle, there is: the Technical Research Center of Finland. It's government owned, and contract work can be obtained at a relatively high cost, particularly for a small company. The food and confectionery industries have so many different products, many with relatively small markets and each with its own problems; they have been very happy with the help we've been able to give them. I can't yet give you a figure on the increased sales.

A. P. G. Kieboom - You referred to sucrochemistry. Do you also conduct research on other applications for sucrose besides food?

Ahvenainen - At the moment, our group works only on food applications, although we'd like to work on all the areas where sugar can be used.

D. E. Webster, B.C. Sugars - How widespread is the use of your "natural sugar" logo? You mentioned it's used on packages and literature to customers; what about other uses--automobile decals, tie tacks--or do you do that sort of thing?

Kuusisto - It is quite widespread. Our aim is to get the logo on our customers' packages.

Enrique R. Arias, Sugar Cane Growers Cooperative of Florida - Do you have a total cost for the program, including advertising? Have you measured the attitude change in the consumer, from the beginning of the program until today?

Kuusisto - The attitude change is measured continuously, at least every six months. As I mentioned in the paper, we are now at the level of acceptance of wheat flour and are approaching honey. Our sales have shown growth over the past two years.

Ahvenainen - The costs of advertising are many times the costs of the product application research.

PRODUCTION AND CHARACTERIZATION OF DEXTRAN FROM SUGARCANE JUICE

Arthur W. Miller and Frederick W. Parrish
U.S. Department of Agriculture

Margaret A. Clarke
Sugar Processing Research, Inc.

INTRODUCTION

Secretary Block's Challenge Forum 1984 "New Uses for Farm Products" (U.S. Dept. Agriculture, 1984) focused on the development of new uses for farm products in order to stimulate the growth of this nation's farm economy. Included in the many ideas and suggestions was a need to identify new uses of existing crops leading to enhanced profits. The Agricultural Research Service Program Plan (U.S. Dept. Agriculture, 1985) establishes a high priority need in commodity conversion and delivery. The plan recognizes the need for "knowledge of ways to apply new and emerging technologies to convert surplus commodities and their derivatives into competitive or novel products which can open new foreign and domestic markets, displace imports, or add value." The need for new uses to give added value products to sugarcane, its constituents, and by-products is the objective of a current research project by Southern Regional Research Center and Sugar Processing Research, Inc.

In terms of chemical resources, and particularly of renewable chemical resources, refined cane sugar is a high volume, high purity product, widely available at a relatively low price (assuming 20-25 cents/lb). It is therefore reasonable to consider refined cane sugar as an inexpensive fermentation substrate, and, of course, raw cane sugar or molasses would be even less expensive to use. It can be expected that fermentation products would command a price of the order of \$1/lb, thus meeting the added-value objective. The current objective is to develop improved fermentation processes for production of enzymes and polysaccharides for food applications in order to provide added value to cane sugar juice.

It was decided to focus initially on the production of dextrans from cane sugar juice because sucrose is the unique carbon source for the formation of this polysaccharide (Alsop, 1983). This is because dextran is synthesized by bacterial enzymes, primarily extracellular, that will accept only sucrose as their substrate. In addition, dextrans are known to occur in a range of size and shape (or structure), resulting in products possessing viscosity and gelling properties suitable for food applications (Sandford et al., 1984; Glicksman, 1982). The dextrans of potential use in food applications would possess a molecular weight in the range of 60,000-90,000 daltons. They are tasteless and odorless

materials which are chemically inert and compatible with most food ingredients. In addition, dextrans can function as emulsifiers and can stabilize oil-in-water emulsions. They have humectant properties and impart body to liquid systems (Glicksman, 1982).

Many dextrans are completely metabolized, but slowly, which suggests their use in low-calorie foods and in reducing diets. While dextran is not on the U.S. Food and Drug Administration's list of substances Generally Regarded As Safe (the GRAS list), due to the lack of the mandatory toxicological studies, it was on this list prior to 1977, and food applications have been both proposed (Glicksman, 1982) and patented (de Belder, 1985). In the Philippines, a dessert of water-insoluble dextran flavored with fruit syrups is known as NATA. In the mid-1960s, food-grade dextran commanded a price of about \$1.60 per lb, and clinical dextran of \$14.75 per lb (Jeanes, 1966).

A critical need in World War II for blood volume expander spurred the development for this use of clinical dextran, made by fermentation of sucrose to dextran using Leuconostoc mesenteroides. This product was made in a sugar refinery by Refined Syrups and Sugars, Yonkers, NY. Clinical dextran was used effectively in the Korean War, and remains a major end use of dextran (de Belder, 1985; Alsop, 1983; Glicksman, 1982). The research and development for the production of dextran is one of a number of successful accomplishments of the fermentation group at the Northern Regional Research Center, U.S.D.A., Peoria, IL.

Applications of dextrans and dextran-derived products, increasingly in biotechnology and related areas, resulted in the production of as much as 70,000-100,000 tons worldwide in 1980 (Yalpani, 1985). As a class, water-soluble polysaccharides for food, general industry, and oil and gas recovery are estimated to be a \$500 million market by 1990 (Parmenter, 1986).

The bacteria that can produce dextrans when grown on sucrose-containing media are members of the genera Leuconostoc, Streptococcus, and Lactobacillus (Hucker and Pederson, 1930). The largest number of dextrans of different structural types are produced from sucrose by species of Leuconostoc, so it is this genus which was initially studied most (Jeanes et al., 1954). The involvement of streptococcal glucans in dental caries has resulted in an increasing amount of research on dextrans from Streptococcus (Hamada and Slade, 1980). The amount and type of dextran produced depend on the individual strain and on the culture conditions employed (Alsop, 1983; Sidebotham, 1974; Jeanes, 1965).

In western nations, dextran is commercially produced almost exclusively from the bacterial strain Leuconostoc mesenteroides NRRL B-512F, which has been selected for high dextransucrase production. In addition, this strain secretes only a single dextransucrase and a low amount of levan-sucrase. The dextran derived from this strain has the fewest non-1,6 linkages; i.e., linkages other than α -1,6 linkages. Consequently, this dextran is only slightly branched, which is desirable for clinical usage (de Belder, 1985). The side chains are attached to the α -1,6

backbone by α -1,3 linkages. The length of the side chains is one glucose for 45% of the branches, two glucose units linked α -1,6 for 40%, and more than two glucose units, all joined α -1,6, for the remaining 15% of the branches (Larm et al., 1971).

Our experimental approach is to study the parameters involved in the formation and isolation of dextrans of various structural types by fermentation of cane sugar juice with Leuconostoc mesenteroides in order to improve yields and lower production costs of food-grade products. This paper is a presentation of our experimental results to date.

We have so far characterized dextran and other fermentation products primarily by their behavior on an ion-exchange column. We propose to determine physical properties of these dextrans by conventional methods; e.g., by using a Brookfield instrument for viscosity and an Instron for gel strength (Descamps et al., 1986).

In addition, we expect to be able to correlate the structures of the dextrans with physical properties relating to functionality in food formulations. Structural analysis of the various dextrans has been facilitated by the application of Fourier transform infrared difference spectroscopy (Seymour et al., 1980) and ^{13}C -nuclear magnetic resonance spectroscopy (Seymour and Knapp, 1980a and b). Although these physical techniques require somewhat larger amounts of material, they are more rapid than conventional methylation analysis (Seymour et al., 1977).

In future work, we also intend to examine continuous culture methods for dextran production in order to improve product uniformity and reduce reactor volume. Relatively little work has been done on such methods for dextran production (Alsop, 1983). We also intend to explore the production of dextran by purified dextransucrase, the enzyme that actually synthesizes the dextran, which it does by direct transfer of glucose residues from sucrose to dextran. There are several advantages to using purified dextransucrase. One is increased flexibility of reaction conditions. This would permit an increased rate of dextran production and would make it easier to control the molecular size and polydispersity (\bar{M}_w/\bar{M}_n) of the dextran (Paul et al., 1986).

Another advantage to the use of purified dextransucrase is that no sucrose is lost to nonproductive bacterial growth and metabolism, so that the sucrose can be converted almost completely into just two products, dextran and fructose. A third advantage is that purification can remove undesirable enzyme impurities, such as levansucrase. It may also be possible to suppress levansucrase production selectively (Jacques, 1985).

The feasibility of the purified-enzyme approach has been established by the continuing work of Monsan and coworkers. Large quantities of dextransucrase enzyme have been produced by fed-batch or continuous culture of Leuconostoc mesenteroides (Monsan and Lopez, 1981; Paul et al., 1984), and large-scale purification of the enzyme has been accomplished by phase partition (Paul et al., 1984; Walter and Johansson, 1986).

Our work to date has only examined fermentation conditions. No attention has yet been given to the genetic manipulation of Leuconostoc bacteria producing these enzymes. Genetic approaches have promise, as shown by recombinant DNA work done on the genes responsible for dextran production by Streptococcus bacteria (Perry et al., 1985; Gilpin et al., 1985; Robeson et al., 1983).

EXPERIMENTAL - MATERIALS AND METHODS

Organisms. Isolates of Leuconostoc mesenteroides (NRRL B-512F and NRRL B-1208) were obtained from the U.S.D.A. collection at the Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL.

Fermentations. Filter-clarified, heat-sterilized cane juice was inoculated with 0.02 volume of inoculum, which was the dextran production medium described by Jeanes (1965), but containing 2% sucrose rather than 10% sucrose.

HPLC analysis. High pressure liquid chromatography was conducted on a Waters Associates (Milford, MA) Model 6000 A solvent delivery system, with a R-401 Differential Refractometer detector. The column, an Aminex HPX-87C (Bio-Rad Laboratories, Richmond, CA), contained an ion-exchange resin in the calcium form. It was preceded by a Bio-Rad deashing pre-column and was eluted with water. The column was kept at 85°C during chromatography.

To identify peaks, culture samples were spiked with standard compounds before chromatography. Cultures were also subjected to thin-layer chromatography on silica gel using acetonitrile-water mobile phases (Gauch et al., 1979). This distinguished sucrose and isomaltose, which migrated almost identically on the HPLC column. Compounds were quantified by integrating the peaks from the HPLC refractometer. Dextran T2000 from Pharmacia was used as the standard for dextran quantification.

RESULTS AND DISCUSSION

Fig. 1 shows the time course of product formation by L. mesenteroides NRRL B-512F in cane juice buffered with 30 mM potassium phosphate to give a starting pH of about 6.5. (In commercial dextran production, the initial pH is roughly 7.) The final products are shown in the bottom panel. Other products of the fermentation not shown in Fig. 1 are lactic and acetic acids (which are removed by the deashing column), carbon dioxide, and ethanol. Ethanol elutes under the trailing edge of the mannitol peak on the column used in Fig. 1, and small amounts are therefore difficult to detect. Mannitol and fructose are potentially valuable by-products of the fermentation.

Fig. 1 shows glucose, the oligosaccharides leucrose (α -D-glucopyranosyl-(1 \rightarrow 5)-D-fructopyranose), isomaltose, and isomaltotriose, and

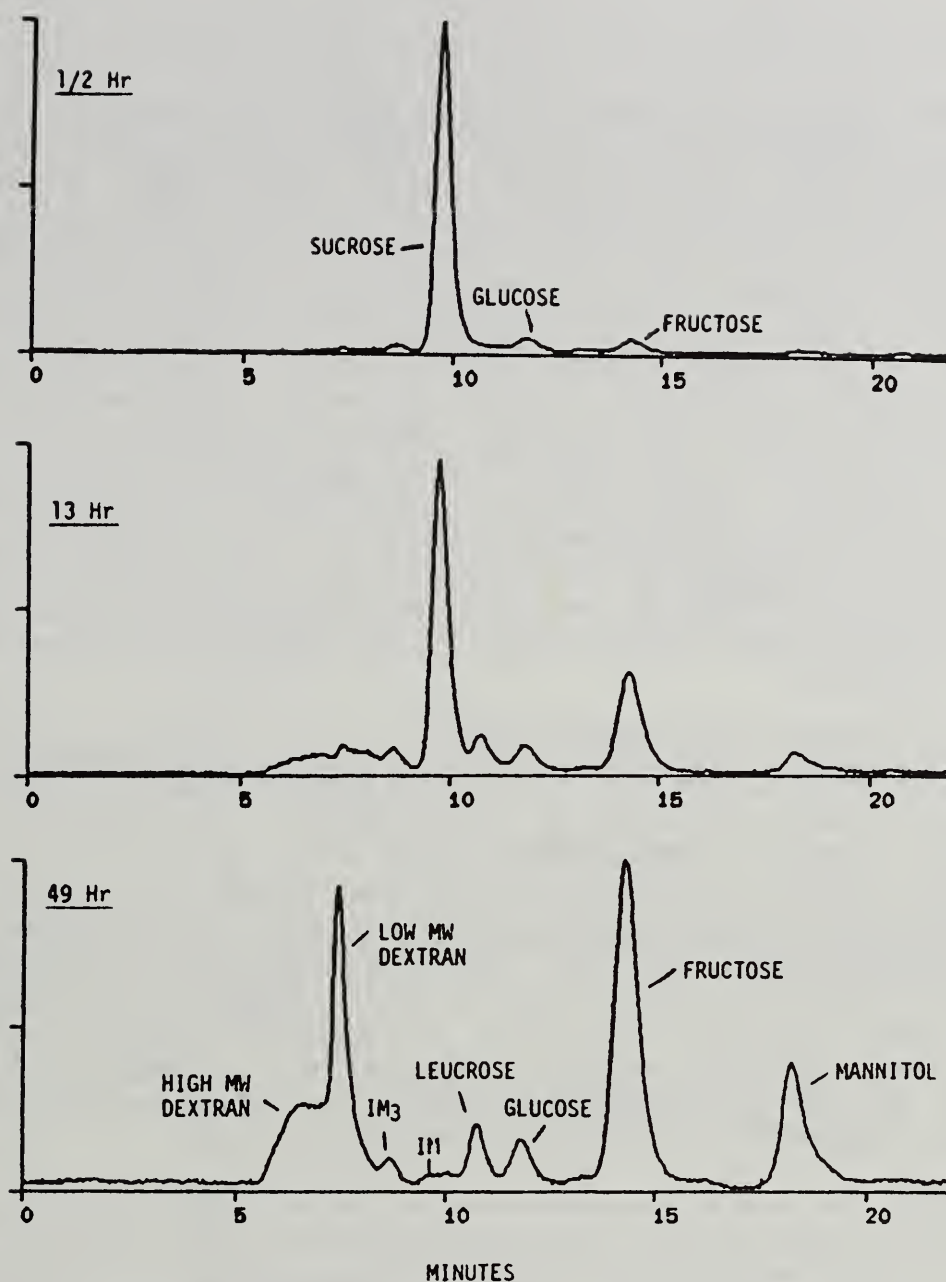


Figure 1--Time course of product formation from cane juice with *Leuconostoc mesenteroides* NRRL B-512F, as shown by HPLC on a calcium-form ion-exchange column. IM and IM₃ are isomaltose and isomaltotriose, respectively.

two peaks labeled "high mw dextran" and "low mw dextran." Dextran-producing fermentations result in a highly polydisperse dextran; i.e., the dextran has a broad molecular weight distribution. As well as being broad, both fractional ethanol precipitation and size exclusion chromatography show that this distribution is distinctly bimodal. On this basis, dextran can be characterized as being of "high" or of "low" molecular weight. Culture conditions affect the average size of both high and low molecular weight dextran, and so the boundary between the two is somewhat arbitrary. For our purposes, low-molecular-weight dextran will be defined as having a molecular weight of 5000 or less. By this definition, only the high-molecular-weight fraction is commercially valuable. Optimum culture conditions will therefore minimize the formation of low-molecular-weight dextran. Glucose and oligosaccharides (principally the disaccharide leucrose) are also produced, both in whole-cell culture and by purified dextransucrase acting on sucrose, and these represent additional losses at the expense of high-molecular-weight dextran.

If the pH of the juice was not altered, little dextran was produced by the B-512F strain, and over 70% of the sucrose remained after two days (Fig. 2A). This turned out to be a pH effect. The addition of 10 mM potassium phosphate, adjusted to give a starting pH of 6.5, resulted in more cell growth, almost complete sucrose consumption, and the formation of dextran in an amount quite similar to that of conventional, commercial fermentations (Fig. 2B). The pH profiles for the cultures shown in Figs. 2A and B are given in Fig. 3. With the addition of phosphate, longer times are spent in the best pH regions for growth, enzyme production, and enzyme reaction with sucrose, as described by Alsop (1983). Without the addition of phosphate, the pH rapidly dropped down to where the bacteria stop growing or metabolizing.

Increasing the phosphate concentration above 10 mM or the pH above 6.5 resulted in only slight further improvements. That phosphate itself was not a critical addition was found by substituting the non-metabolized buffering compound PIPES (piperazine-N,N'-bis[2-ethanesulfonic acid]), which has the same pK_a as phosphate. Results were nearly the same as with an equal concentration of inorganic phosphate. The choice of buffering agent may not be completely arbitrary, however. Ammonium compounds have been reported to drastically lower dextransucrase production by L. mesenteroides NRRL B-512 (Tsuchiya et al., 1952).

The low level of dextran production in unmodified juice seems to contradict the well-known, rapid thickening of contaminated juice. One explanation might lie in the fact that the B-512F strain did not originate from cane juice, but from a bottle of infected root beer. Strains isolated from cane juice might be expected to give higher levels of dextran.

We have not found this to be true. Fig. 4 shows the products obtained from several strains on filtered but otherwise unaltered juice. Fig. 4B shows the small amount of dextran given by strain B-512F. Fig. 4C shows the improvement given by merely thawing a juice sample and letting it

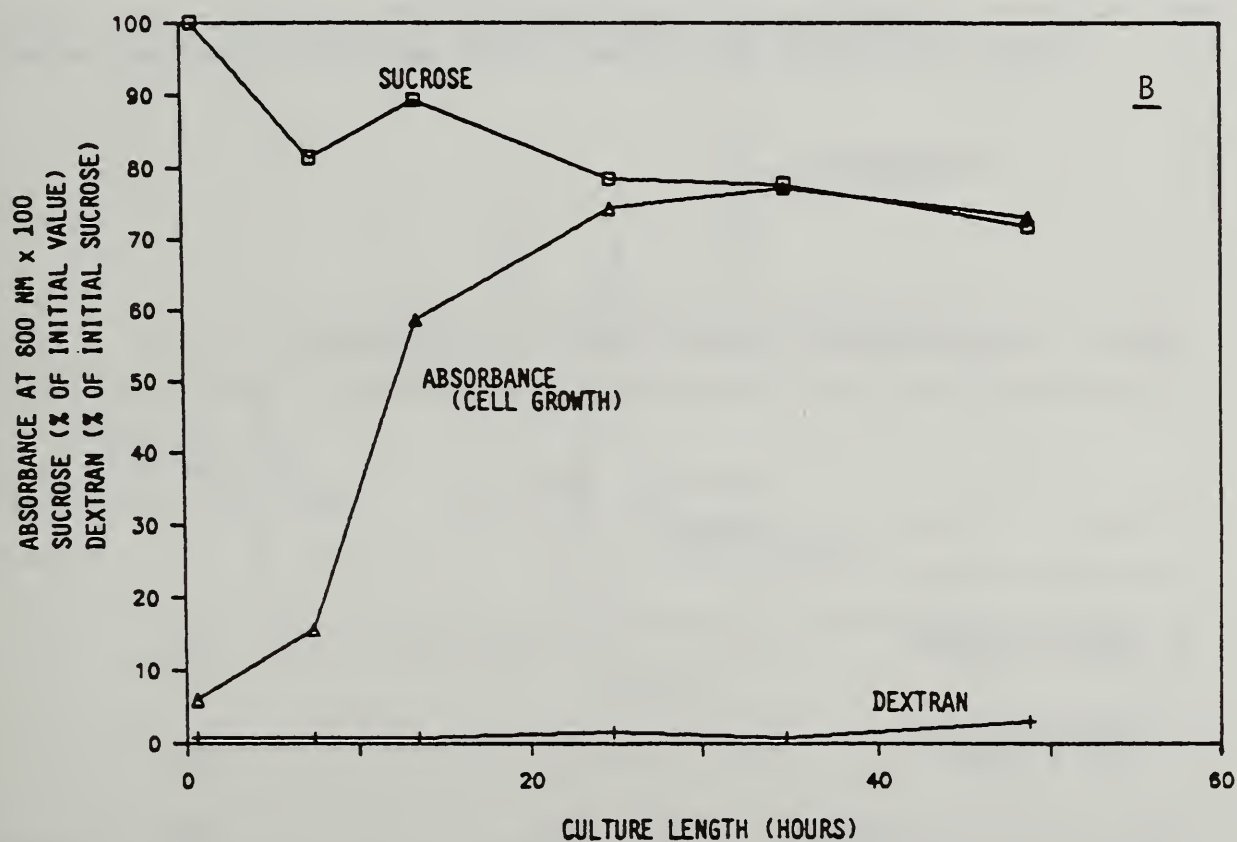
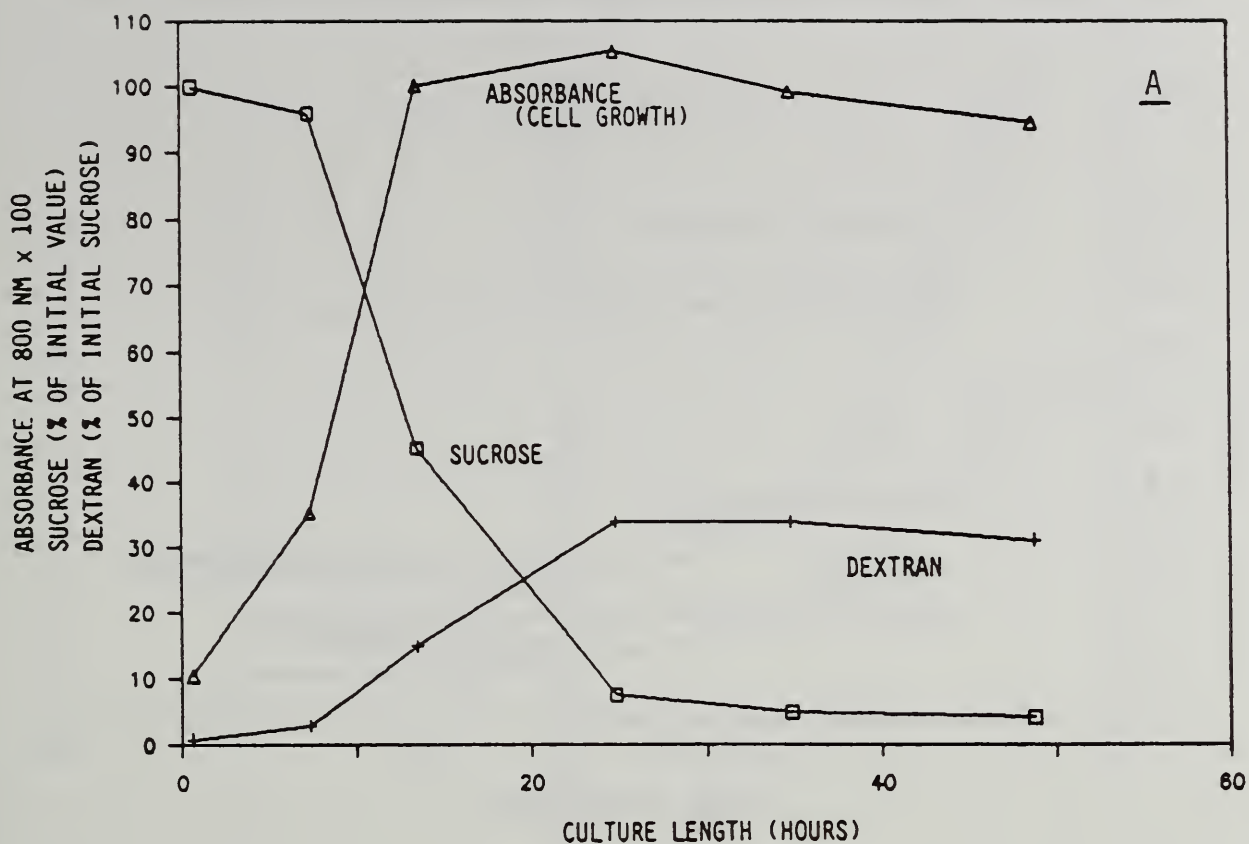


Figure 2--Growth of *Leuconostoc mesenteroides* NRRL B-512F on cane juice. (A) Unmodified juice. (B) Juice with 10 mM potassium phosphate added.

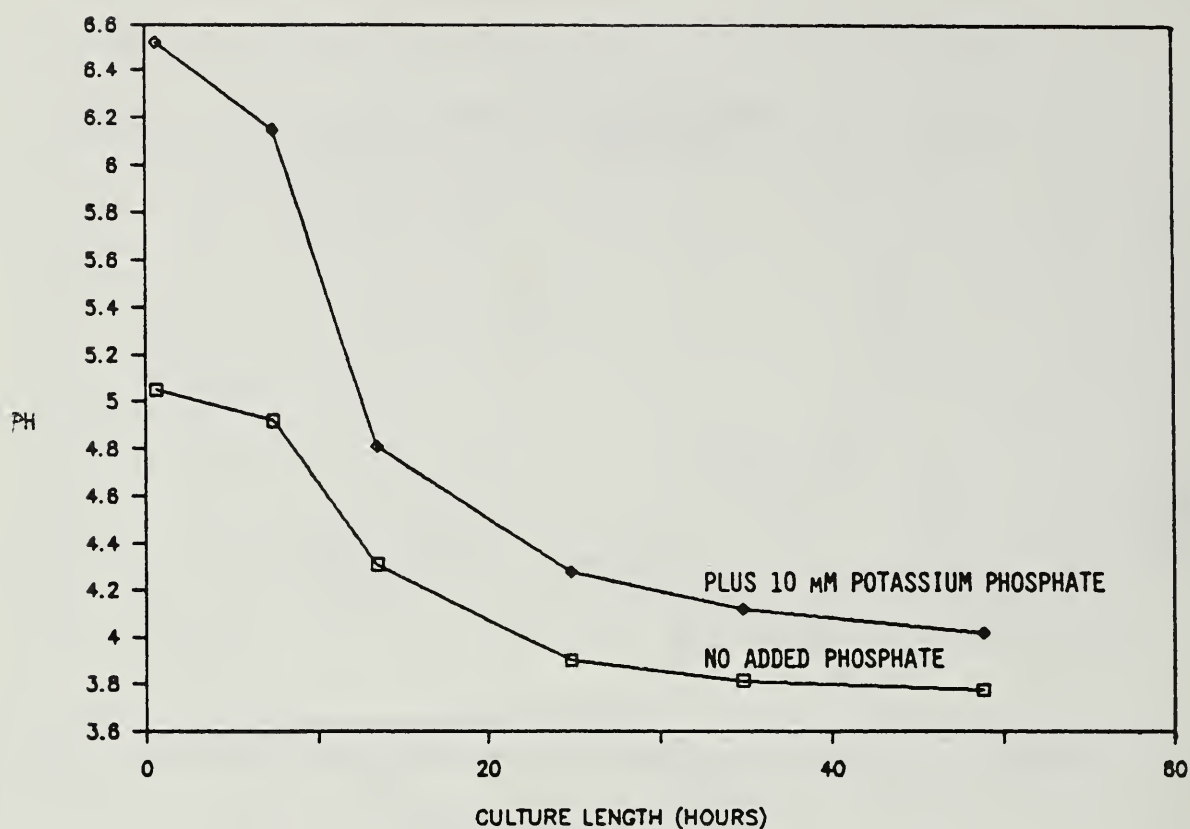


Figure 3--Growth of Leuconostoc mesenteroides NRRL B-512F on cane juice. Change in pH with and without 10 mM potassium phosphate added.

Table 1--Comparison of strains grown on cane juice.

Strain	"Dextran" yield, w/v (low MW + high MW)	Fraction of dextran with high molecular weight
<u>L. mesenteroides</u> NRRL B-512F	3-1/2 - 4-1/2%	High
<u>L. mesenteroides</u> NRRL B-1208	2 - 2-1/2%	Low
Unidentified isolate from cane juice	3-1/2 - 5%	Low

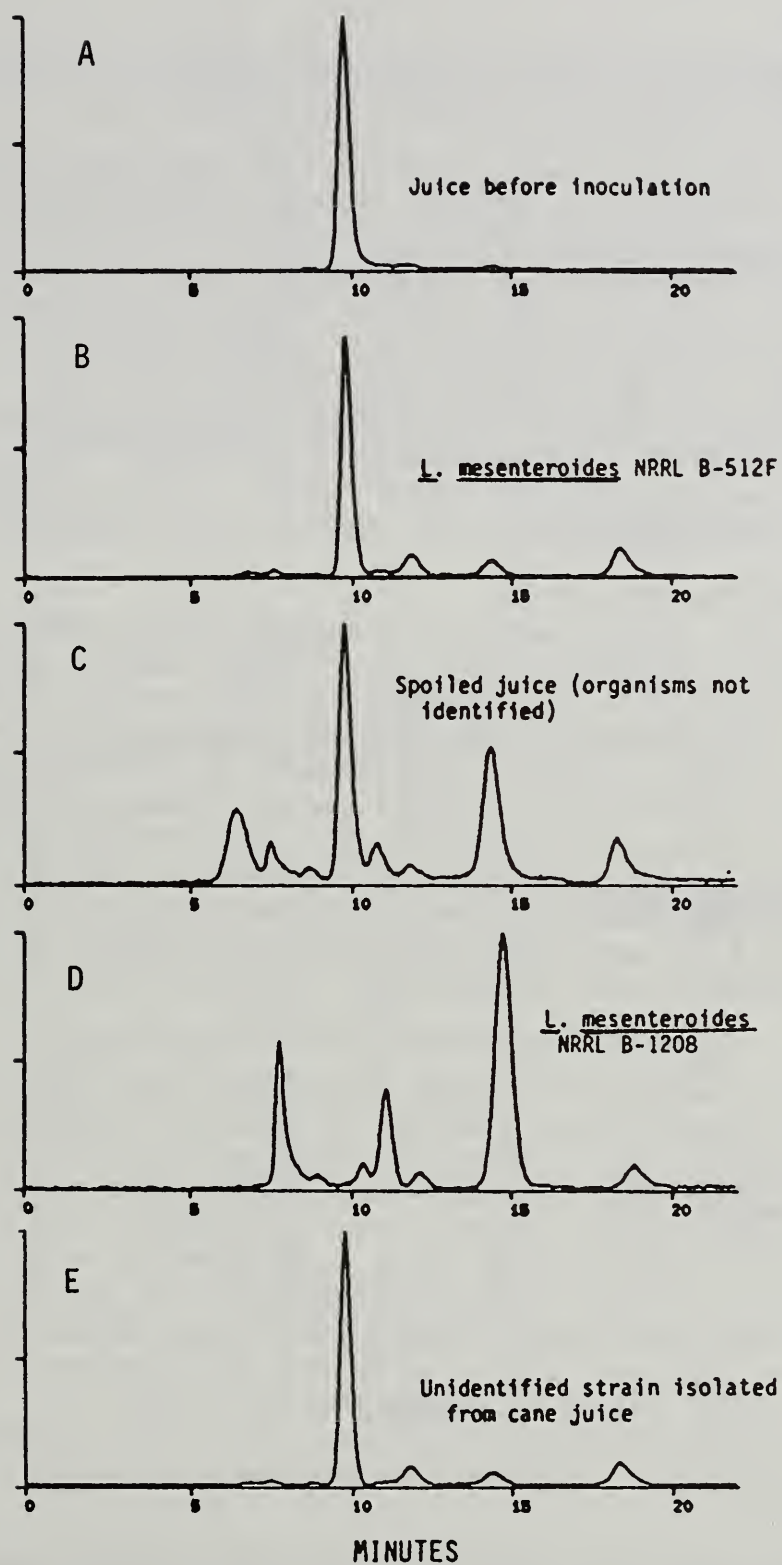


Figure 4--Final products of several dextran-producing organisms grown on filtered cane juice. Shown are HPLC chromatograms from a calcium-form ion-exchange column.

sit at room temperature. Even in this case, however, sucrose consumption stopped with 25-30% of the sucrose remaining.

Figs. 4D and E show that strains isolated from cane juice cannot be relied upon to be superior to B-512F. Strain B-1208, isolated from cane juice and producing a lightly branched dextran very similar to that of B-512F, converts all the sucrose and gives a respectable amount of dextran (Fig. 4D). However, this dextran almost all belongs to the undesirable low-molecular-weight fraction. A large amount of another undesirable product, the disaccharide leucrose, is also made. The pH dropped more slowly for the B-1208 culture than for the others, indicating slow growth, and this may be responsible for both the complete conversion of sucrose and the high dextran yield. Fig. 4E shows that a strain isolated from cane juice gives results much like those from B-512F.

The amounts of low- and high-molecular-weight dextran varied with both strain and culture conditions (Fig. 5). In this figure, arrows are placed over the peaks for high-molecular-weight dextran, low-molecular-weight dextran, and leucrose. Two strains isolated from cane juice (Figs. 5C and D) show relatively high amounts of low-molecular-weight dextran, but the high-molecular-weight dextran present is better resolved from the low-molecular-weight fraction than for strain B-512F (Fig. 5A), and probably represents higher molecular weight polysaccharide than the high-molecular-weight region in Fig. 5A. The two strains isolated from cane juice also show high amounts of leucrose (Fig. 5C) or glucose (peak to the right of the right-most arrow in Fig. 5D).

Fig. 5B shows the results for strain B-512F when the juice was diluted with an equal volume of water (before the addition of phosphate). The ratio of high- to low-molecular-weight dextran is greater than without dilution (Fig. 5A). This is a well-known effect from conventional fermentations: the overall percent yield of dextran from sucrose and the fraction of the dextran that is of high-molecular weight both increase as the sucrose concentration decreases. The amount of leucrose decreases. (These effects are offset, however, by increased fermentation time and by the cost of the higher volume of solvent needed to precipitate an equal weight of polysaccharide, which is of course very dilute for low initial sucrose concentrations.) The presence of glucose and fructose also affect the yield of high-molecular-weight dextran by decreasing it.

A summary of the most important chromatographic data is given in Table 1. The percentages shown for strain B-512F represent dextran yields from cane juice that are comparable to those obtained by conventional commercial fermentation. Two reservations should be considered in the interpretation made here of the chromatographic data shown. First, the peaks labeled as dextran also contain some levan. Second, the quantitative division of high-molecular-weight dextran from low-molecular-weight dextran is much more arbitrary than it would be by size exclusion chromatography, for example. We have performed some size ex-

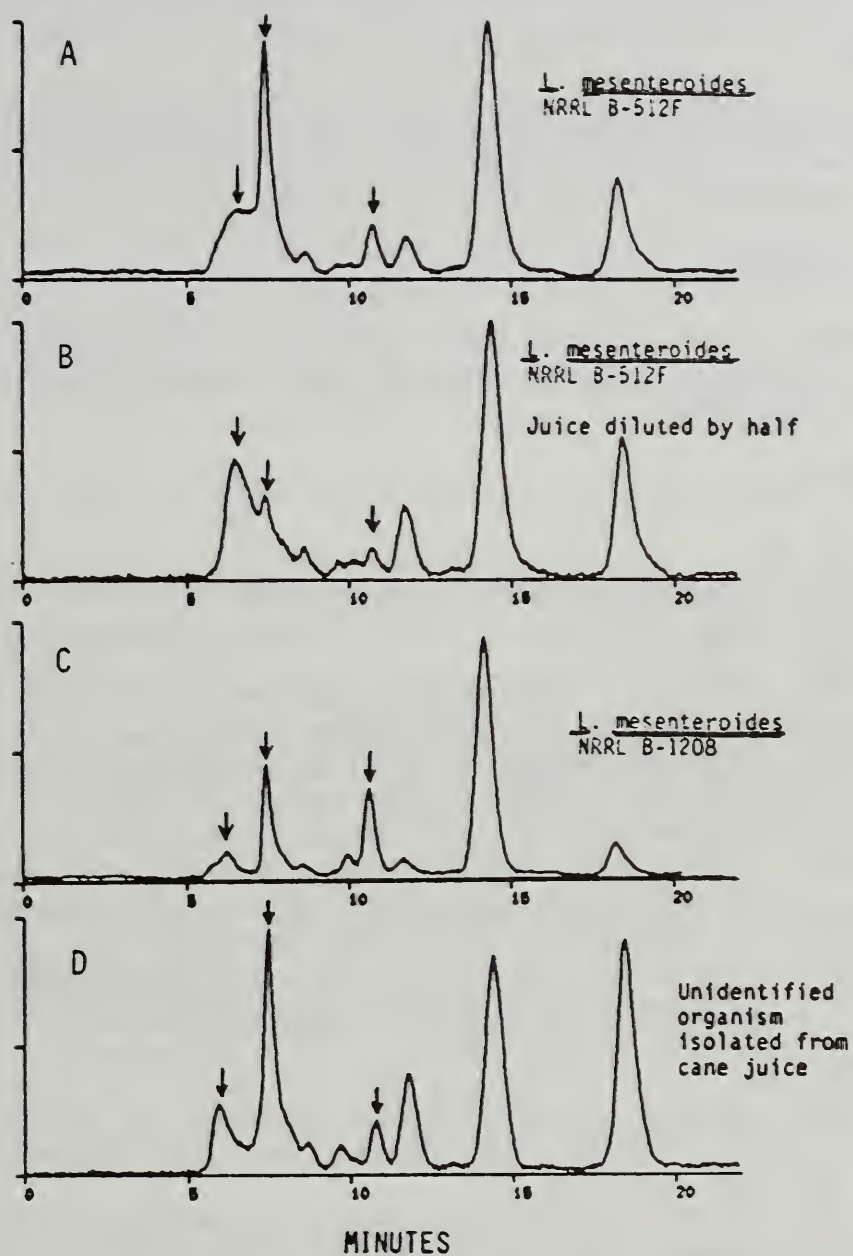


Figure 5--Final products (50 hr) in phosphate-buffered juice. Shown are HPLC chromatograms from a calcium-form ion-exchange column. On each chromatogram, the three arrows mark the positions of (left to right) high-molecular-weight dextran, low-molecular-weight dextran, and leucrose.

clusion chromatography on these culture samples, but our data are as yet very limited. Considerable data obtained by size-exclusion HPLC relating to commercial dextran-producing fermentations by strain B-512F are given in a review on dextran production by Alsop (1983).

SUMMARY

Dextran can be produced from cane juice in yields similar to those obtained on conventional industrial media. The only modification of the juice required is an increase in its pH.

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DISCUSSION

R. Riffer, C and H Sugar - You said that the dextransucrase is produced both cell-bound and extra-cellularly. Does this mean that some of the dextran is extracellular and some is formed as the capsule? If so, what is the distribution?

Miller - To break this down into classes of glucan-producing enzymes: One class makes a soluble glucan--what we are used to thinking of as dextran. Another class makes glucans that are considerably less soluble. The enzyme that forms soluble glucans is mostly secreted; apparently the enzyme that produces insoluble dextrans is cell-bound. The material produced is not so much a traditional capsule, but a matrix in which the bacterium becomes embedded--a protective adaptation, in a way.

Riffer - So the production you are talking about is extracellular?

Miller - That's correct; for the B512F strain, essentially all the enzyme is secreted extracellularly.

A.P.G. Kieboom, Delft University - I remember a presentation by Pierre Monsan last year. He came up with a two-stage process to make dextrans, with some regulation of molecular weight. First, lower molecular weight dextrans were produced, and these were subsequently used as the base for the second stage of production. Have you also tried this approach?

Miller - Basically, yes, but the problem is to make the size fraction of interest in the yields that you need. Pierre Monsan has been working on this for many years with improving results.

The purified dextransucrase can be used in a similar process, adding low molecular weight material to grow higher molecular weight dextran. A lot of that work was done as early as the 1950's at the Northern Regional Research Center of the U.S. Department of Agriculture, in Peoria, Illinois. That's a continuing research project--it is possible to take that approach.

Kieboom - In your case, where you have about 10% of low molecular weight dextran, is it possible to put that back in the growth medium and have them grow again?

Miller - They will grow, to a certain extent. But the final mixture is liable to be even more polydisperse and of low molecular weight. But that is a worthwhile approach--an excellent point.

Jaap Bruijn, Sugar Milling Research Institute - Can you give me a rough idea of the total financial value of current dextran production and prospects in the U.S. market?

Miller - There is a wide range of figures in the literature--\$40 million is one that's often quoted--but we have no really reliable figure.

STUDIES OF THE COLOUR OF U.K. BEET WHITE SUGAR

N.W.Broughton, D.Sargent, B.J.Houghton and A. Sissons

British Sugar plc, Research Laboratories

1. INTRODUCTION

This paper presents a summary of the work undertaken by the Research Laboratories of British Sugar plc from 1982 to the present time on the nature and origins of colour in beet white sugar.

The research programme has as its ultimate objective the development of least cost methods of controlling white sugar colour to meet market requirements at the time of despatch. We aim to know how to control colour in the process so that sugar can be manufactured to the appropriate colour specification at least cost and how to control storage so that the sugar colour at the time of despatch is that which the customer requires. The fundamental methodology underlying our colour studies is to work backwards from white sugar colour through the successive massecuites and liquors which may give rise to white sugar colour.

During the extraction of white sugar from beet, British Sugar uses a Dorr defeco-carbonatation juice purification system for the simultaneous liming and carbonatation of raw juice. In the sugar end of the factory a 3-boiling crystallisation system is employed, with raw (2nd product) and affinated after-product (3rd product) sugars being returned to thick juice prior to crystallisation of white sugar from the standard liquor so formed. This white sugar has, on average, a colour of 19 ICUMSA units.

Throughout this paper, it should be noted that 'colour' and various quantitative expressions for it all refer to absorbance of light having a wavelength of 420nm, unless specifically stated otherwise.

2. THE NATURE OF SUGAR COLORANTS

In the first part of our work (Shore et al. 1984) an understanding was developed of the nature and location of the colouring matter in our white sugar. This was achieved with the aid of a low pressure gel permeation chromatographic fractionation technique.

Colorant concentrates isolated from the surface and from the inside of white sugar crystals were fractionated on Sephadex G-25. As can be seen from Figure 1, which is typical of the G-25 fractionations of colorants isolated from samples of British Sugar white sugar, the colorant concentrate from the inside of the crystal contained material of molecular weight up to 5,000 daltons. Colorants on the surface of the crystal had significantly lower molecular weights, only up to about 1,000 daltons.

Our observations for the molecular weight of beet sugar colorants agree with those of Prey and Andres (1973) and have subsequently been supported by Vukov et al. (1984).

In cane sugars, there is a divergence of views concerning the absolute size of the colorant molecules present in sugar. Certain researchers (Buganenko et al. 1971, Smith et al. 1981) report colorants with molecular weights up to about 5,000 daltons while others (Roberts and Godshall 1978, Yamane et al. 1968, Smith 1966) report colorants with substantially higher molecular weights.

There appears to be general agreement, however, that, in both beet and cane sugars, the relatively high molecular weight colorants are preferentially included within the sugar crystal while the lower molecular weight colorants are found on the crystal surface (Tu et al. 1977, Reva et al. 1977, Matvienko et al. 1985, Roberts & Godshall 1978, Smith et al. 1981, Smith 1976). Certain workers have identified the occluded colorants as melanoidins and the excluded colorants as being caramel types (Prey and Wesner 1975, Kozyavkin et al. 1980, Saber-Guda et al. 1979). A possible explanation for the inclusion of specific compounds within the sugar crystal has been proposed (van der Poel et al. 1986, Verhaart et al. 1967) on the basis of molecular weight. Mantovani et al. (1985a) have reviewed the findings of many workers on the behaviour of different types of colorant with possible mechanisms and factors affecting the incorporation of colour into the white sugar crystal during crystallisation.

Based upon our current knowledge (Shore et al. 1984), we have no evidence to show that colorants derived from phenolic compounds in the beet make a significant contribution to white sugar colour. This view is also held by Reinefeld et al. (1982) but Madsen and co-workers (Madsen et al. 1978, Kofod-Nielsen

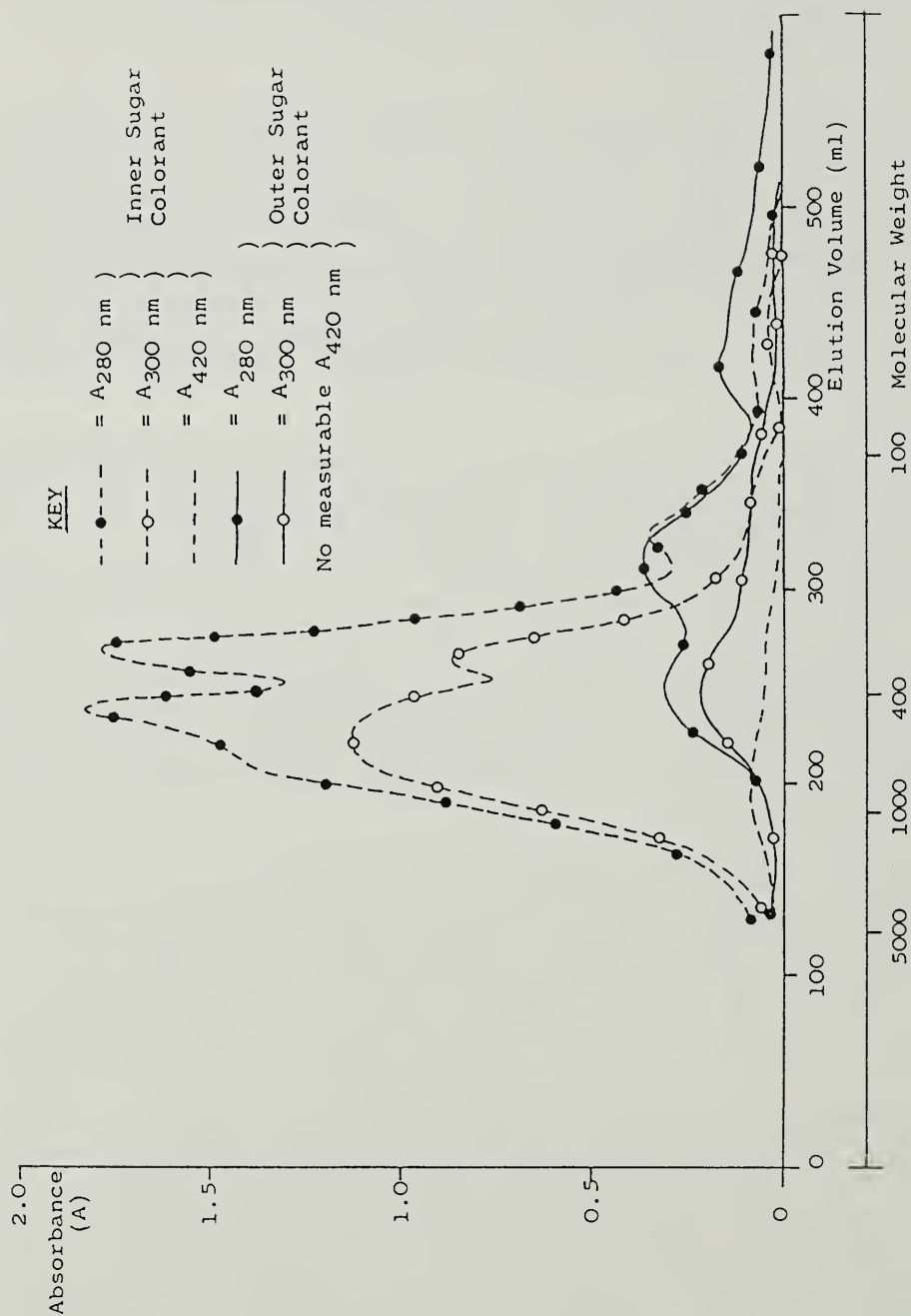


Figure 1 G-25 fractionation of sugar colorants

et al. 1980) consider that colour formation caused by polyphenolic compounds in beet and juices is a main factor influencing colouring substances entering the sugar crystal. This may be associated with the DDS system of juice extraction and purification. In the cane industry, colorants derived from phenolic and flavonoid compounds are generally considered to make significant contributions to white sugar colour (Tu 1974). The role of plant colorants in relation to other classes of colorant in cane sugar is reviewed by Clarke et al. (1985).

3. EXPERIMENTAL RESULTS

3.1 Distribution of Colour Through the White Sugar Crystal

With regard to the location of colour (irrespective of type or mechanism of inclusion) in the white sugar crystal, our early studies (Shore et al. 1984) using an ethanol/water mixture washing technique demonstrated that, as Figure 2 shows, colour could be increasingly removed by dissolving increasing proportions of the crystal up to, on average, a maximum of about 25% reduction by removal of 20% of the sugar crystal. As continued washing, and hence solution of more of the crystal, had little further effect in reducing the colour of the sugar, we term this "internal" colour 'irreducible'.

Current investigations using solutions of mineral water sugar (9 ICUMSA units colour and 0.002% ash) as the washing agent have demonstrated a similar pattern of colour distribution in white sugar (Figure 3). A similar colour distribution in white beet sugar has recently been reported by van der Poel et al. (1986) who also observe an increase of colour around the nucleus of the crystals. This phenomenon, which we have also observed on occasion, may indicate occlusions incorporated in the period during or shortly after seeding or may be the result of release of mother liquor as conglomerated crystals are dissolved.

Similar proportionate distributions of the total colour were found (Shore et al. 1984) in both high colour (>30 ICUMSA units) and low colour (ca. 15 ICUMSA units) sugars. Hence, a high colour white sugar does not contain exceptionally large proportions of the total colour in the surface layer; as with lower colour sugar, the majority of colour is distributed through the inner 75 to 80% of the crystal.

It was also demonstrated that the reactions forming colour during storage of white sugar occur throughout the crystal (Shore et al. 1984) since the distribution of colour in the crystal did not change after storage. In Figure 4, sugar stored at 50°C had increased in colour by 20 ICUMSA units (+106%) while a duplicate sample stored at ambient

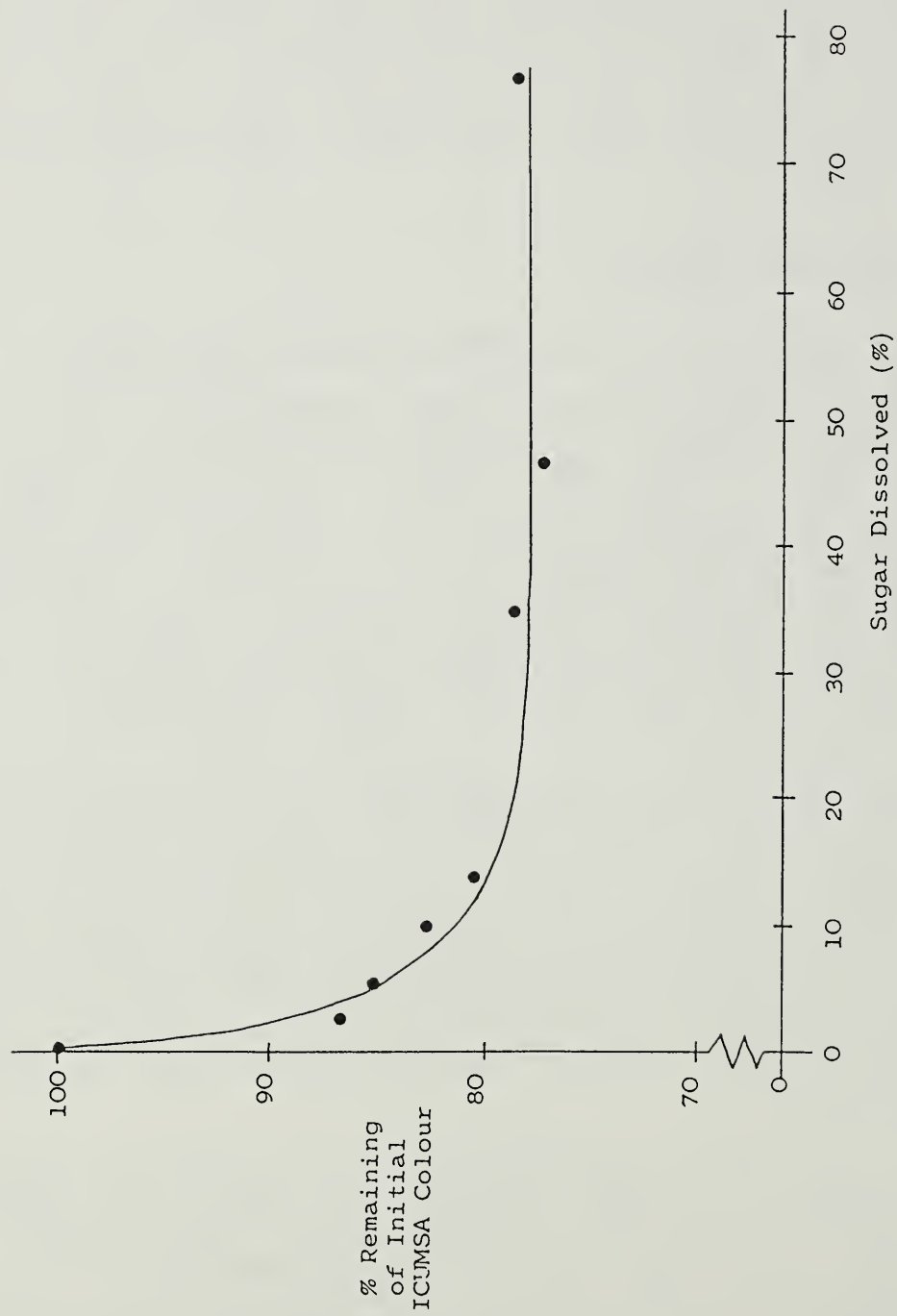


Figure 2 Colour distribution in production white sugar -
washing with ethanol/water mixtures

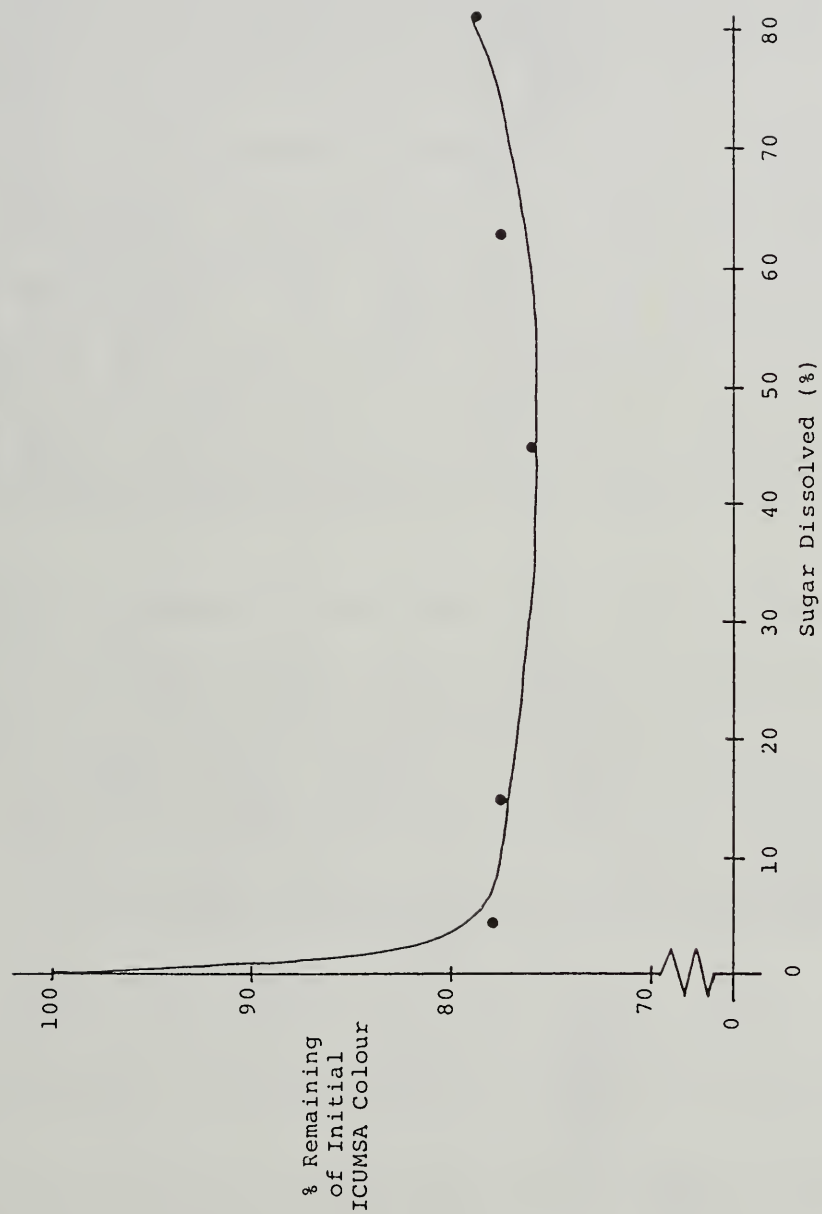


Figure 3 Colour distribution in production white sugar - washing with mineral water sugar syrups

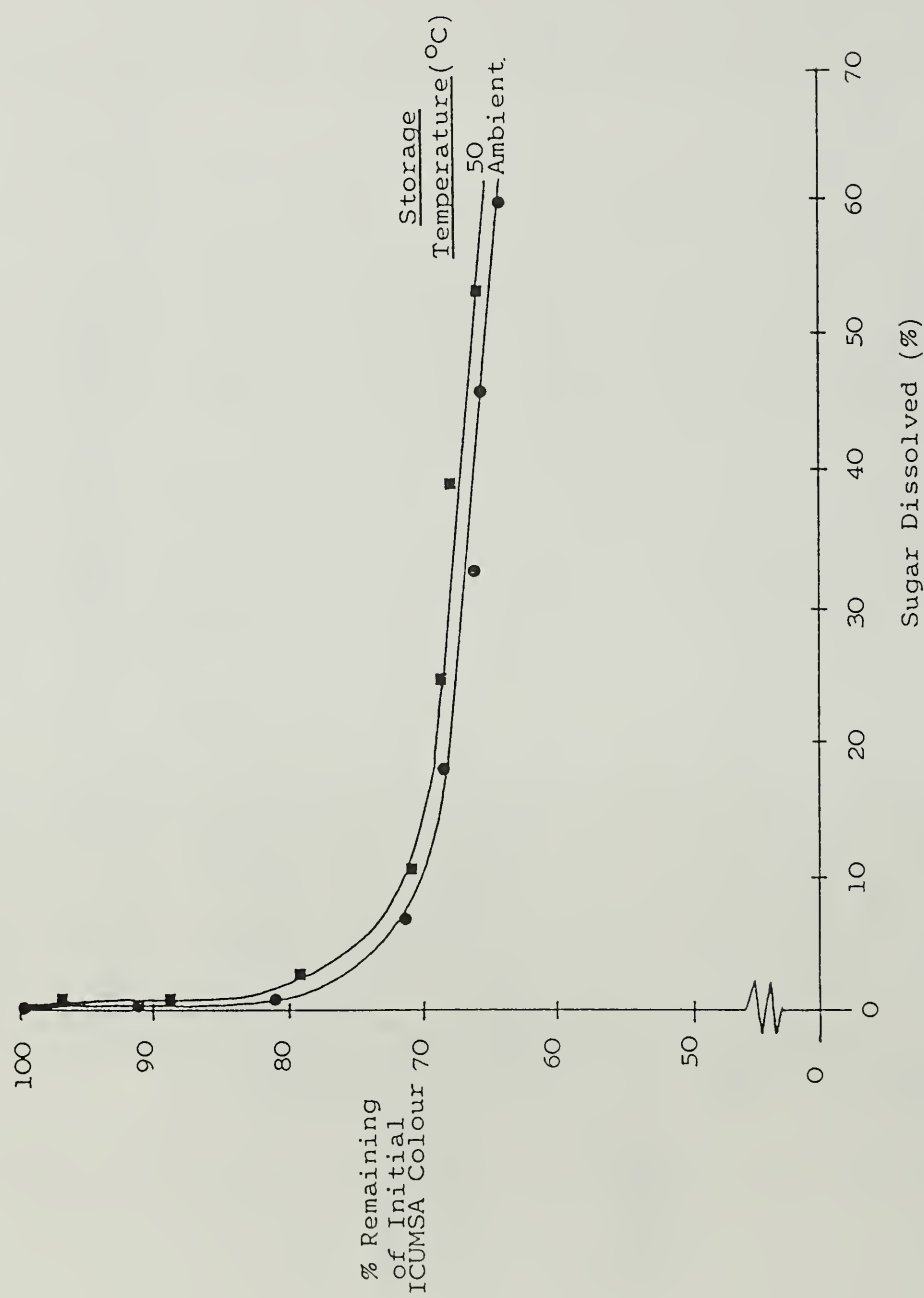


Figure 4 Colour distribution of production white sugar stored at room temperature and 50°C

temperature had increased by less than 1 ICUMSA unit (+4%). Washing of these sugars showed that the colour of the sugar stored at 50°C could not be reduced to that of the lower colour sugar : although more colour was removed in the initial washings of the higher colour sugar, on a percentage proportional basis the colour removed as the crystal was dissolved was the same for both sugars.

Our observations on colour distribution in white sugar coupled with the data on molecular weights of colorants in the sugar suggest that the high molecular weight compounds are responsible for most of beet white sugar colour.

3.2 Colour Distribution in Raw and After-Product Sugars

The distribution of colour in beet raw and after-product sugars (typical colours being about 1,000 and 4,000 ICUMSA units respectively) was very different from that in white sugar as Figures 5 & 6 demonstrate. In these cases, about 80-85% of the sugar colour is susceptible to removal by washing. Upon washing with mineral water sugar solutions, these sugars attain their 'irreducible' colour level after about 10% of the sugar has been dissolved. van der Poel et al. (1986) report similar observations with Dutch white sugars.

3.3 Colour Formation in the Process - Use of a Colour Balance

To identify the sources of white sugar colour through the process, with a quantitative appreciation of their relative significance, we have developed the colour balance introduced in an earlier paper (Shore et al.1984).We have reported (Broughton et al.1986) our detailed findings and calculations with special attention to the sources of standard liquor colour and to the extent to which colour is formed in the sugar end and recycled to the white pan stage.

The underlying concept of the colour balance is of a 'quantity' of colour, calculated by multiplying the tonnes of any particular liquor or sugar in process by its percentage refractometric dry matter content (RDS) and its ICUMSA colour, thus:

$$\text{'Quantity' of colour} = \frac{\text{Tonnes of juice or sugar}}{\text{or sugar}} \times \frac{\% \text{RDS}}{100} \times \text{ICUMSA colour}$$

The results of such calculations, which are numerically large relative to 100t beet sliced, are not actual quantities, of course, hence the use of 'quantity'. These calculations enabled us to study how colour moves from stage to stage of the factory process and where it is created within the

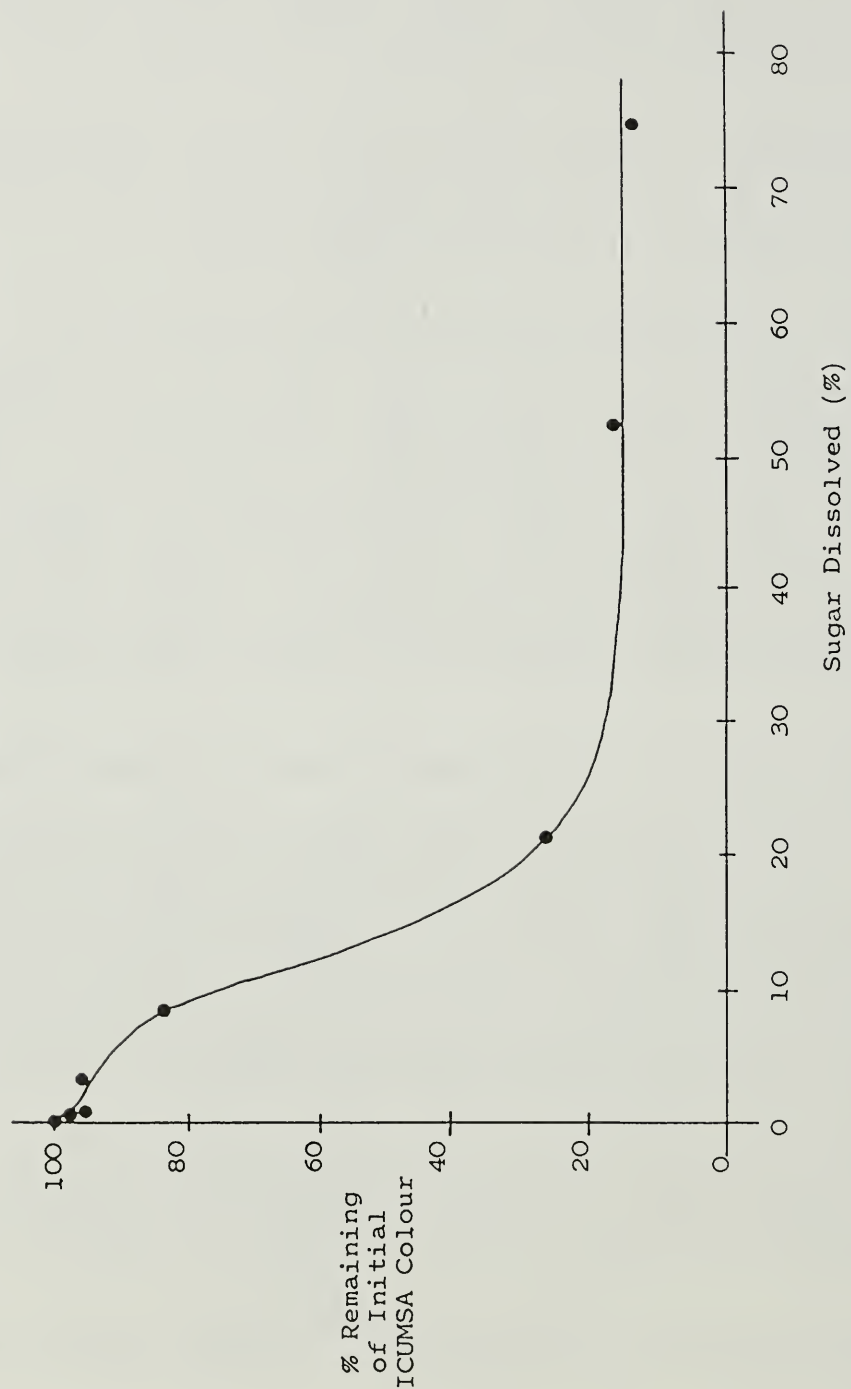


Figure 5 Colour distribution in raw sugar

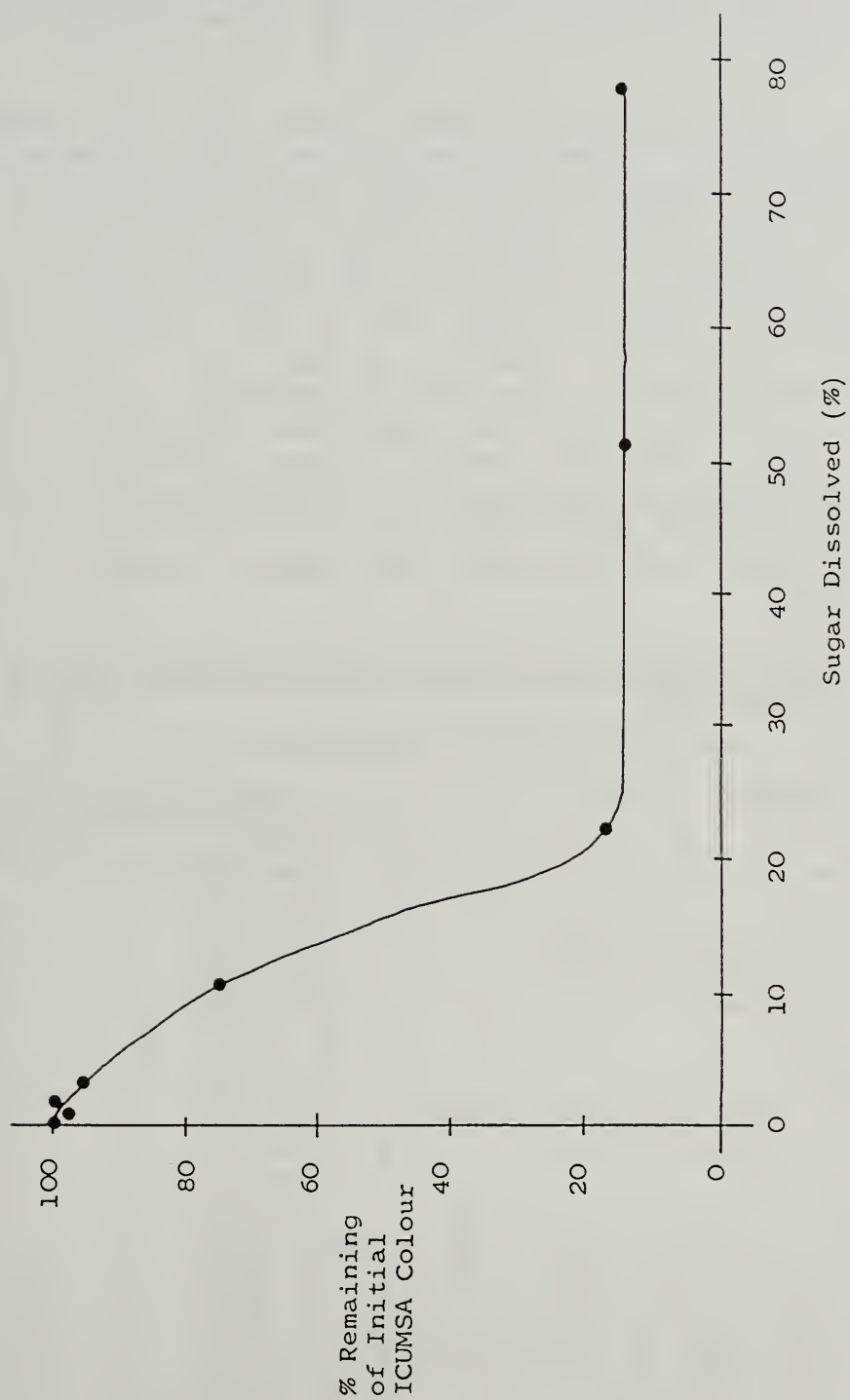


Figure 6 Colour distribution in after-product sugar

process. During the 1984/85 sugar beet campaign, colour balances were constructed for 7 factories; one of these factories (D) was studied both at the beginning and at the end of campaign. The average and range of ICUMSA colours measured on the juices and sugars from these factories are given in Table 1.

Using these ICUMSA colours and mass balance and colour balance computer programs, the magnitudes of the colour increases in the successive stages of the process were calculated (Table 2). A specimen colour balance print-out taken from the computer is presented in Table 3. The increase for any one stage is expressed as a percentage of the input colour for that stage. For example, there was a 14% average colour increase in the raw pans (Table 2) so that, for a 'quantity' of colour in high green to raw pans of 5619 units, the average 'quantity' of colour in the raw massecuite was 5619×1.14 , i.e. 6406 units. The 11% average colour increase in the subsequent operations associated with centrifuging the raw sugar would increase the average 'quantity' of colour in the centrifuge output (raw sugar and low green) to 6406×1.11 , i.e. 7111 units.

Table 1 ICUMSA colours of factory products during 1984/85 campaign
(Data from 7 factories)

Product	ICUMSA Colour (I.U.)	
	Range	Average
Thin Juice	936-2526	1670
Thick Juice	1057-3386	1994
Unfiltered Standard Liquor	1444-3123	2050
Filtered Standard Liquor	1455-3095	2080
White Massecuite	1534-3106	2245
White Sugar	11.5-27.4	18.7
Wash Syrup	1358-4105	2813
High Green Syrup (ex centrifugals)	3370-6980	5054
High Green Syrup (ex tank)	3008-7095	4692
Raw Massecuite	4174-8239	5816
Raw Sugar	344-1827	971
Low Green Syrup (ex centrifugals)	9083-16823	11437
Low Green Syrup (ex tank)	6952-17664	11309
A.P.Massecuite (into crystalliser)	9447-30940	16060
A.P.Massecuite(out of crystalliser)	11494-37926	20760
After-Product Sugar	1380-10798	3704
Molasses	18981-61059	34460
Affination Magma	2387-11533	6386
Affinated After-Product Sugar	320-2890	1122
Affination Syrup	4372-27138	14696

Table 2 Average Colour Increases - 1984/85 Campaign (Data for 7 factories)

% Colour Increase	Factory							Range	Arithmetic Mean
	A	B	C	D	D	E	F	G	
Evaporators	22	14	35	35	58	20	14	52	31
Standard Liquor Tanks, Heaters & Melter/Dissolver	25	20	6	27	22	5	18	-2	15
White Pans	1	13	8	6	11	7	-6	13	7
White Centrifugals	-2	-2	-5	6	-15	2	3	10	0
High Green Syrup Tanks and Heaters	0	0	0	0	19	0	0	8	3
Raw Pans	13	19	2	15	2	17	32	9	14
Raw Centrifugals	3	19	8	25	26	1	-3	8	11
Low Green Syrup Tanks and Heaters	0	0	0	0	25	0	0	6	4
After-Product Pans	27	94	26	68	18	33	46	50	45
After-Product Crystallisers	74	28	24	16	20	22	23	36	30
After-Product Centrifugals	3	-3	12	7	-4	-1	-5	0	1

Table 3 Specimen colour balance calculation

SUGAR END MASS AND COLOUR BALANCE (WITH WATER AFFINATION; AFFINATION SYRUP RETURNED TO LOW GREEN TANK.)									
Tonnes/day	% on beet	% RDS	% Purity	Colour (I.U.)	Colour "Tonnes"/1000	Colour Summary	% INCREASE		
THIN JUICE	11721.86								
THICK JUICE EX EVAPS.	2593.64	24.17%	91.90	1579	2813	S.L. TANKS & HEATERS	1		
THICK JUICE TO STORE	711.43	6.63%	91.80	2407	4276	(filters/dissolvers)			
FLASH EVAP. WATER	5.50	0.05%				WHITE PANS	13		
JUICE TO SUGAR END	1876.71	17.49%							
CONC. REF. SUGAR	90.13	0.84%	91.80	2407	3094	WHITE CENTRIFUGALS	10		
RAW SUGAR TO DISSOLVER	397.68	3.71%	100.00	100	9				
AFF'D A.P. TO DISS.	198.77	1.85%	99.00	857	337	H.G. TANKS & HEATERS	8		
DISSOLVER DILUTION	265.40	2.47%	99.00	2304	449	RAW PANS	9		
STANDARD LIQUOR UNF.	3607.47	33.62%	93.23	2226	5767	RAW CENTRIFUGALS	8		
STANDARD LIQUOR FILT.	3607.47	33.62%	93.23	2302	5964				
WATER EVAPORATED	817.67	7.62%				L.G. TANKS & HEATERS	6		
WHITE MASSECUIE	2789.80	26.00%	94.12	2598	6731				
WHITE SUGAR	1117.62	10.42%				A.P. PANS	50		
HIGH GREEN AS SPUN	1095.17	10.21%	86.73	23	26				
WASH SYRUP	778.78	7.26%	93.94	6152	5379	A.P. CRYSTALLISERS	36		
WASH WATER	201.77	1.88%		3353	2008	A.P. CENTRIFUGALS	0		
A.P. SUGAR TO RAW PANS	0.00	0.00%	97.00	7532	0				
HIGH GREEN TO RAW PANS	1054.92	9.83%	86.73	6672	5619	THICK JUICE TO			
LOW GREEN TO RAW PANS	0.00	0.00%	75.96	16290	0	MOLASSES FACTOR	4.41		
WATER EVAPORATED	202.20	1.88%							
RAW MASSECUIE	852.72	7.95%	87.38	7717	6130	WHITE MASSECUIE TO			
RAW SUGAR	397.68	3.71%	99.00	857	337	WHITE SUGAR RATIO	261.86		
LOW GREEN AS SPUN	532.14	4.96%	75.49	14968	6297				
WASH WATER	77.11	0.72%							
HIGH GREEN TO A.P. PANS	40.25	0.38%	86.73	6672	214	Calc. Colours ("Tonnes"/1000)			
LOW GREEN TO A.P. PANS	567.34	5.29%	75.96	16290	7307	STD. LIQUOR INPUT	5898		
WATER EVAPORATED	101.40	0.95%				WHITE MASS. OUTPUT	7413		
A.P. MASS. TO CRYST.	506.19	4.72%	76.68	23408	11252	RAW MASS. INPUT	5619		
A.P. MASS. EX CRYST.	224.87	2.10%	97.00	31759	15266	RAW MASS. OUTPUT	6635		
MOLASSES	344.30	3.21%	59.15	7532	1677	A.P. MASS. INPUT	7521		
WASH WATER	62.98	0.59%		52882	13646	A.P. MASS. OUTPUT	15323		
A.P. SUGAR TO AFFINATION	224.87	2.10%	97.00	7532	1677	AFFINATION INPUT	1677		
WATER TO AFFINATION	17.11	0.16%				AFFINATION OUTPUT	1069		
AFFINATION MAGMA	241.98	2.26%	97.00	10205	2272				
AFFINATION SYRUP	36.38	0.34%	83.00	22281	620				
AFFINATED SUGAR	198.77	1.85%	99.00	2304	449				
WHITE PAN YIELD	45.83%	sugar in massecuite							
RAW PAN YIELD	56.16%	sugar in massecuite							
A.P. PAN YIELD	58.59%	sugar in massecuite							

As Table 2 shows, on average, particularly large percentage colour increases were found to occur in evaporation, after-product pans and after-product crystallisers. Other workers (van der Poel et al. 1986, Hangyal and Paradi 1984, Faviell 1980) have reported their studies on areas of colour formation in the beet process although not in as much detail as presented in this paper.

3.3.1 Composition of standard liquor colour

Table 2 shows that the colouring materials present in standard liquor constituted, on average, 93% of white massecuite colour, the remaining 7% being made during the boiling of the white pans. It was considered appropriate, therefore, to determine the origin of standard liquor colour and, in particular, how much was likely to be sugar-end colour, which the earlier work (Shore et al. 1984) had shown to contain relatively large amounts of the high molecular weight molecules considered to make a large contribution to white sugar colour.

In British Sugar factories, standard liquor consists of thick juice plus the following recycled sugar-end products : wash syrup, raw sugar and after-product sugar (which may have been affinated). The colour of standard liquor comprises that present in these sugar-end returns plus the colour present in thick juice, which itself consists of the colour present in thin juice and any colour formed during evaporation, plus any colour formed during the production of standard liquor from its components.

Making use of the 'quantities' of colour computed by the colour balance technique (exemplified in Table 3), the contributions to the colour of standard liquor made by its components were calculated for each factory studied. The pie-chart in Figure 7 shows that, on average, 52% of the total 'quantity' of filtered standard liquor colour was from thick juice - 40% coming from thin juice and 12% being formed during evaporation (calculated by difference of thin and thick juice colours). Wash syrup colour usually made the biggest single contribution to colour recycled to standard liquor from the sugar end: on average, almost two thirds of the 'quantity' of colour returned to filtered standard liquor came from wash syrup. Raw sugar and (affinated) after-product sugar each contributed about half the remainder, on average. The recycle of sugar-end materials, therefore, contributes 35% towards the total colour of standard liquor.

The remaining 13%, which could not be accounted for directly by addition of the individual components, is considered to be colour produced in the dissolving system. Possible main causes include heat and retention time in thick juice tanks and heaters and pipes to the dissolver, and the high temperature

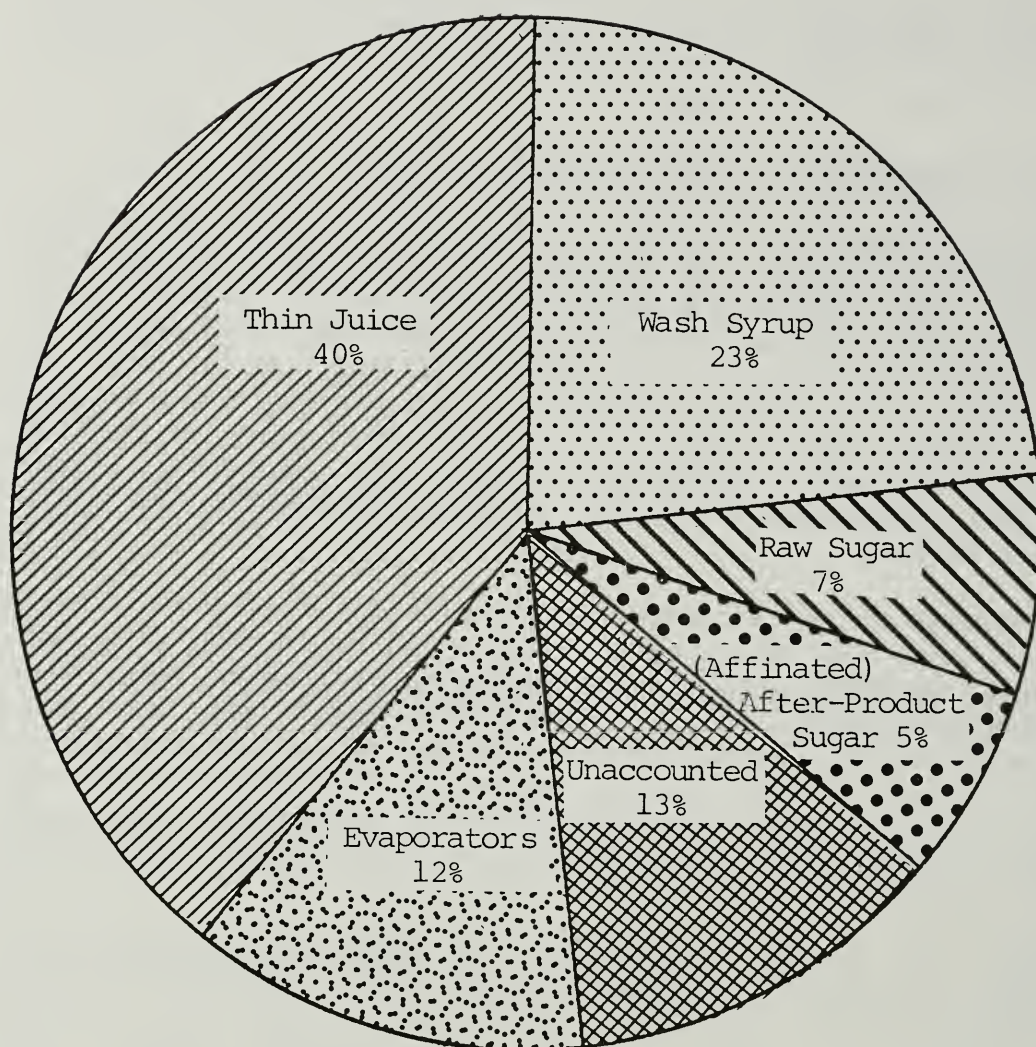


Figure 7 Average contributions to the 'quantity' of colour in standard liquor

of the dissolver. Colour present in minor returns of dilute liquors not otherwise accounted for, such as dissolver make-up water, may also have made a small contribution. Faviell (1980) also considers high melter operating temperatures (ca. 95°C) to be the main cause of colour increases at this stage.

van der Poel et al. (1986) in their studies of the composition of standard liquor colour have also noted that the measured colour of factory standard liquor was greater than the calculated colour from the input materials by about 10%. They also state that, in their experience, standard liquor colour comprises 71% from thick juice, 1.2% from wash syrup, 18.5% from intermediate sugar and 9.2% from affinated after-product sugar.

3.3.2 The origin of standard liquor colour

Although the information in Figure 7 demonstrates which juice or sugar has carried the colouring materials into the standard liquor, it does not necessarily indicate the stage of the process which was the source of the colour. Thus, some of the colour returned from sugar-end materials to standard liquor is thick juice colour which has been recycled rather than passing through the sugar end to be eliminated with the molasses.

In order to estimate the amount of such recycled thick juice colour, the 'quantity' of colour formed by chemical reaction in each of the three boilings was calculated by using the colour balance procedure and by taking the difference between output and input colours for each boiling. The 'quantity' was then partitioned between the materials recycled and those going forward according to the ratio of the masses of those materials. By this means, for any material returned to standard liquor from the sugar end, it was possible to calculate the 'quantity' of colour originating from thick juice as the difference between its total colour and that 'quantity' calculated to have been formed in the sugar end.

Calculation with the average data produced the results shown in Table 4. The rationale behind these calculations is demonstrated in Appendix 1.5 of an earlier paper (Broughton et al. 1986).

Table 4 Colour recycled to standard liquor from sugar end,
average of all factories

Colour recycled from	% Colour Contribution from:		
	Thick juice	Sugar end	Total
Wash Syrup	21	2	23
Raw Sugar	5	2	7
(Affinated) After-Product Sugar	2	3	5
Total	28	7	35

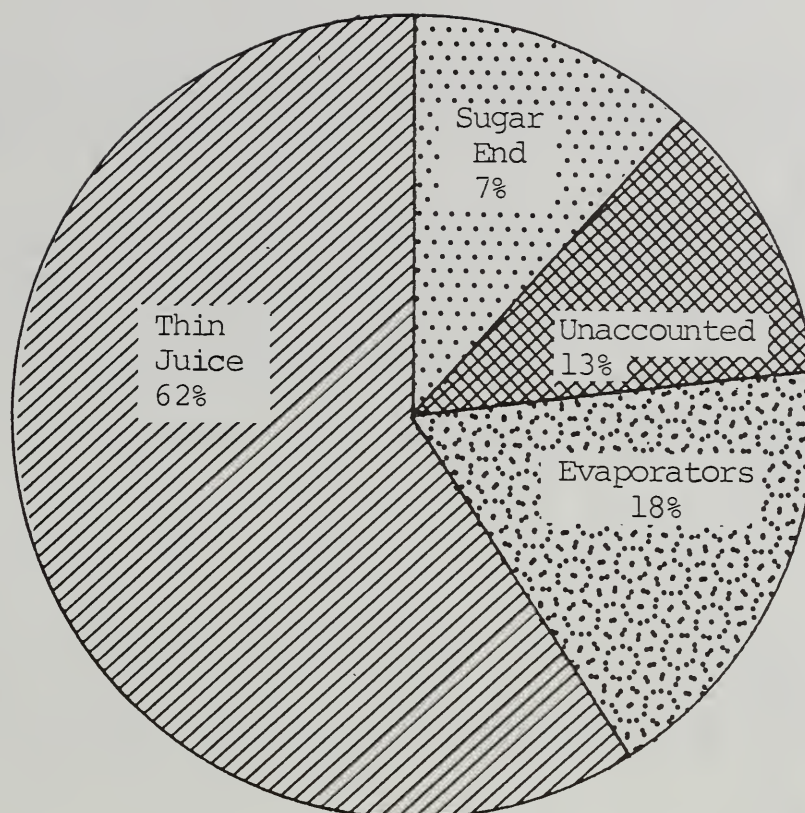
Table 4 shows that, on average, of the 35% contribution made to standard liquor colour by recycled colour from the sugar end, 7% had been synthesised in the sugar end, the remaining 28% being recycled thick juice colour. This means that, on average, less than 10% of standard liquor colour truly originates in the sugar end, 80% originating from thick juice. Of this 80%, 18% was formed during evaporation and 62% was colour present in thin juice, in other words colour from the beet end. This is depicted in the upper part of Figure 8; the lower part is a simplified version of Figure 7, the preliminary analysis of the origins of standard liquor colour. Comparison of the Figures shows that, when recycling of thick juice colour from the sugar end has been allowed for, the average contribution of the 'quantity' of recycled sugar-end colour to the 'quantity' of standard liquor colour is reduced from 35% to 7%.

From Figure 7, the major contribution from wash syrup to colour recycle can be seen. Table 4 shows clearly that much of the colour of wash syrup has its source before standard liquor, and, indeed, wash syrup, raw sugar and (affinated) after-product sugar all return rather similar 'quantities' of sugar-end colour to standard liquor.

Applying the same methodology it was possible to calculate the origin of standard liquor colour for each factory studied. The individual factory results are shown in Table 5 with the average values from Table 4.

The 'quantity' of colour from the beet end prior to the evaporators accounted for between 49 and 74% of the 'quantity' of colour in filtered standard liquor. This contribution was increased by 9 to 29% by the effect of evaporators. Colour made in the sugar end contributed 2 to 14% of standard liquor colour, no one of the three components (after-product sugar, raw sugar and wash syrup) consistently being the main source.

(a)



(b)

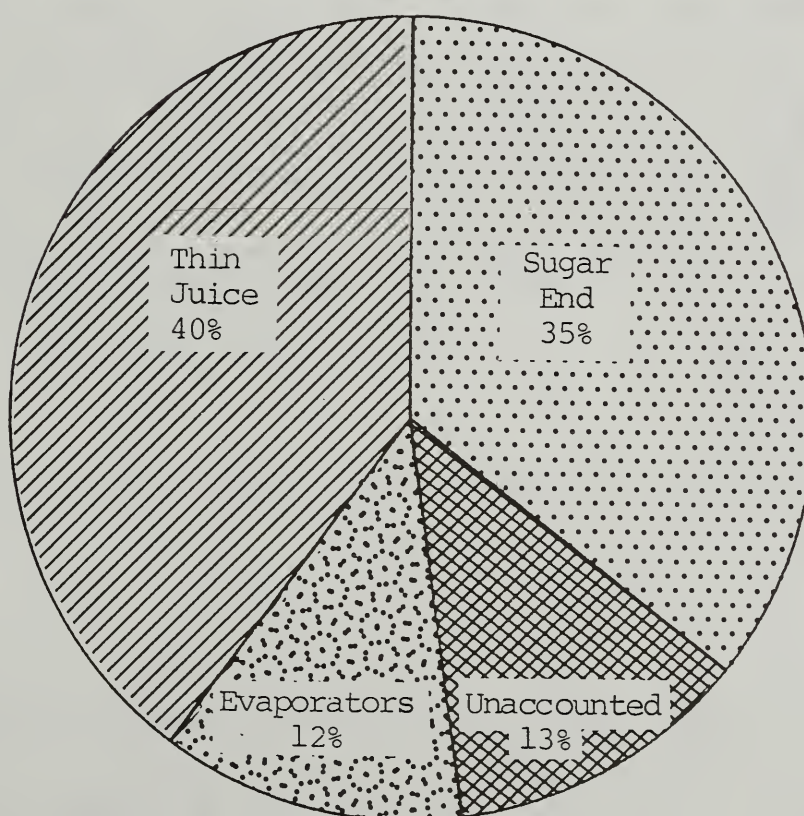


Figure 8 (a) Contributions to standard liquor colour according to origin
(b) Contributions to the 'quantity' of colour in standard liquor

Table 5 % Contributions to Standard Liquor colour according to origin
(Data for 7 factories - 1984/85 Campaign)

Factory	Thin Juice	Evaporators	Thick Juice (Beet-End Colour)	Sugar-End Colour	Wash Syrup	Raw Sugar	After-Product Sugar	Unaccounted Colour
Average	62	18	80	7	2	2	3	13
A	53	12	65	11	0	1	10	24
B	69	9	78	8	0	3	5	14
C	68	23	91	3	1	1	1	6
D	51	19	70	8	2	3	3	22
D	49	29	78	5	0	3	2	17
E	74	14	88	6	3	1	2	6
F	71	9	80	2	0	1	1	18
G	56	29	85	14	6	2	6	1

Unaccounted colour (presumed to be from the dissolving system) accounted for 1 to 24% of standard liquor colour at different factories.

The data presented in Table 5 make plain the differences which exist between factories in the sources of colour. The colour balance approach illustrated here is a powerful tool to apply when considering how best a particular factory should seek to improve its control of white sugar colour.

3.4 Relationship Between Juice Colours and White Sugar Colour

In order to keep below a particular white sugar colour without the need for increased washing, juice colour may have to be kept below a particular value at each stage of the process. Conversely, when juice colours rise there may be an unacceptable increase in white sugar colour unless action is taken. We have therefore examined the relationship between white sugar colour and the colours of juices and massecuite prior to the white pans for the possibility of being able to predict the one from the other.

Elimination factors for colour have been calculated from the ratio of juice ICUMSA colour to white sugar colour at a standard white sugar ash of 0.01% (the maximum target value within British Sugar). This constant ash allows for differences in colour elimination resulting from variables such as amount of white centrifugal wash water which may affect the ash content of the sugar and hence modify the contribution to the overall white sugar colour made by the film of mother liquor around the crystals. Table 6 details average colour elimination factors for selected process materials calculated from factory data during the 1985/86 campaign.

Such factors could form the basis of a computerised alarm and control system for white sugar colour in factories. To illustrate the principle, between second carbonatation juice and white sugar, a colour elimination factor of 85 is a fair 'rule of thumb' (individual factories would use their own values). Thus, to maintain white sugar colour below 20 ICUMSA units (I.U.), second carbonatation juice colour should be below 1700 I.U. unless extra washing or other remedial action is acceptable. Conversely, a second carbonatation juice colour of 2040 I.U. is likely to produce a white sugar colour of 24 I.U. unless remedial action is taken.

Table 6 Average Colour Elimination Factors
(Data for 11 factories, 1985/86 Campaign)

i.e. Juice colour (ICUMSA units)
White sugar colour (ICUMSA units)

Juice	Elimination Factor (at 0.01% white sugar ash)	Range
2nd carbonatation	86	69 - 109
Thin	76	59 - 105
Thick	93	73 - 121
Standard liquor	101	83 - 132
White massecuite	103	88 - 115

Similar colour elimination factors have been reported by Faviell (1980) who comments that significant changes in this factor could indicate specific processing problems, for example, insufficient SO₂ in thin juice. Colour elimination factors of 111 and 113 may be calculated from data given by Bonney and Thomas (1985).

van der Poel et al.(1986) express the elimination of colour as the ratio of the colour in the sugar to that in the juice or massecuite. However, their results are not dissimilar from those reported above.

The elimination of colour in the cane industry is not so great but, of course, the white massecuite colours are much lower. Data given by Bardwell et al.(1981) detail 1st strike massecuite colours of 251 to 431 producing white sugars of 15 to 17 ICUMSA units. The elimination of colour, therefore, ranges from 17 to 25, values considerably less than those experienced in the beet industry.

3.4.1 Relationship between massecuite colour and white sugar colour

Our colour balance work has given us considerable insight into the origins of standard liquor colour and has shown that, on average, the 'quantity' of colour increases by 7% in the white pan. The relationship between the solution colour of the massecuite and the colour of the white sugar which is spun from it is, in many ways, the most crucial of the research questions about colour. We recognise that the colour balance approach sheds little additional light on any selective uptake, by the growing crystals in the white pan, of colour bodies formed in the sugar end in preference to those from the

beet end. We shall be reporting on further studies of this question in later publications.

3.4.2 The elimination of colour from white sugar

The colour present in the feed to the white pans plus that formed during boiling is partitioned between the crystals and liquor not only during the growth of the crystal but also in the centrifuging and washing operations. Some types of colorant (as well as saponins and sulphite) have the property of being positively adsorbed into the crystal structure during boiling (Verhaart et al. 1967, Mantovani et al. 1985a, Keane et al. 1935). For example, colour may bind to the growing surface by physical or chemical adsorption forces and so occur through the crystal. In addition, droplets of mother liquor can be trapped mechanically inside the growing crystals under some conditions of sugar boiling (Mantovani et al. 1985a,b, Guo and White 1983, 1984, Tu et al. 1962, Rossi and Maurandi 1980, Powers 1966). The molecular weight, electrical charge distribution, quantity and type of colorant, and such crystallisation conditions as rate of growth and supersaturation, may modify the location of the colorant in the white sugar crystal.

Crystallisation is a major purification stage but the relatively very high purity of the crystal is offset by the difficulties of removing residual mother liquor. The syrup film remaining on the crystal from the liquor contains some colour which contributes to the overall white sugar colour. Syrup will also be present between the sugar crystals if conglomerates have been formed. A wide particle size distribution (i.e. high coefficient of variation) also militates against efficient syrup removal in centrifuging and washing.

3.4.3 Calculation of the "internal" and "external" components of white sugar colour

Although the mechanisms which determine the residual amounts and locations of ash in white sugar are not necessarily the same as those which determine the residual colour, the parallels are perhaps sufficient to use ash (potassium) data to calculate the proportions of white sugar colour which are "internal" (associated with the crystal itself) and "external" (associated with the syrup film remaining on the crystal surface after washing).

The elimination of non-sugars, which include colouring substances and ash components, from the sugar during the successive steps of pan boiling, centrifugation and washing has been studied extensively (Tu et al. 1977, Smith et al.

1981, Kozyavkin et al. 1980, Mantovani et al. 1985a, Keane et al. 1935, Guo and White 1984, Chiu and Sloane 1980) and both physical and chemical factors have been found to influence the elimination efficiency. It has been shown (Keane et al. 1935, Paine and Balch 1926, Hibbert and Woodwark 1951) that the concentration of non-sugars relative to sugar is very much higher in the surface layers than within the crystal. These authors conclude that the majority of the non-sugars are present in the residual film of syrup on the crystal. It may be significant for this conclusion that the ash contents of the sugars studied were much higher than those of sugars currently produced by British Sugar.

We have found (Shore et al. 1984) that, although the surface concentration is high, the greater proportion of the colour of white sugar is within the crystal. In subsequent work, we have confirmed those observations and found them also true for potassium. The typical washing experiment shown in Figure 9 demonstrates not only that the colour of a sample of white sugar could be reduced only by 25% but that the potassium concentration could be reduced only by 35% of its original value relative to sugar. Similar distribution patterns were observed for colour and potassium whether the sugar was washed with ethanol/water mixtures or mineral water sugar syrups. From a series of such experiments, on average, reductions of only 21% in white sugar colour and 31% in potassium concentration were achievable by washing away progressively more of the crystal.

The presence of about 70% of the total potassium inside the crystal suggests the possible inclusion of quantities of mother liquor from the boiling, and indicates that most of white sugar ash is, like colour, present within the sugar crystal, notwithstanding the high surface concentrations. van der Poel et al. (1986) present similar observations for Dutch white sugars where they report that just over 50% of the ash content is located on the surface of the crystal, i.e. is susceptible to removal by washing.

In view of this parallel distribution of potassium and colour, we have calculated the relative quantitative contributions of "internal" and "external" colour by using the elimination of potassium and colour from measurements on matched pairs of factory white massecuite and white sugar taken at the centrifuge. The potassium elimination factor represents the elimination of the residual syrup and its non-sugars - including some colour - from the crystals during crystallisation, centrifugation and washing. On the assumption that all the potassium is "external", the amount of "external" colour associated with the residual syrup film after these operations may be estimated by dividing the colour of the massecuite by the potassium elimination factor. Once

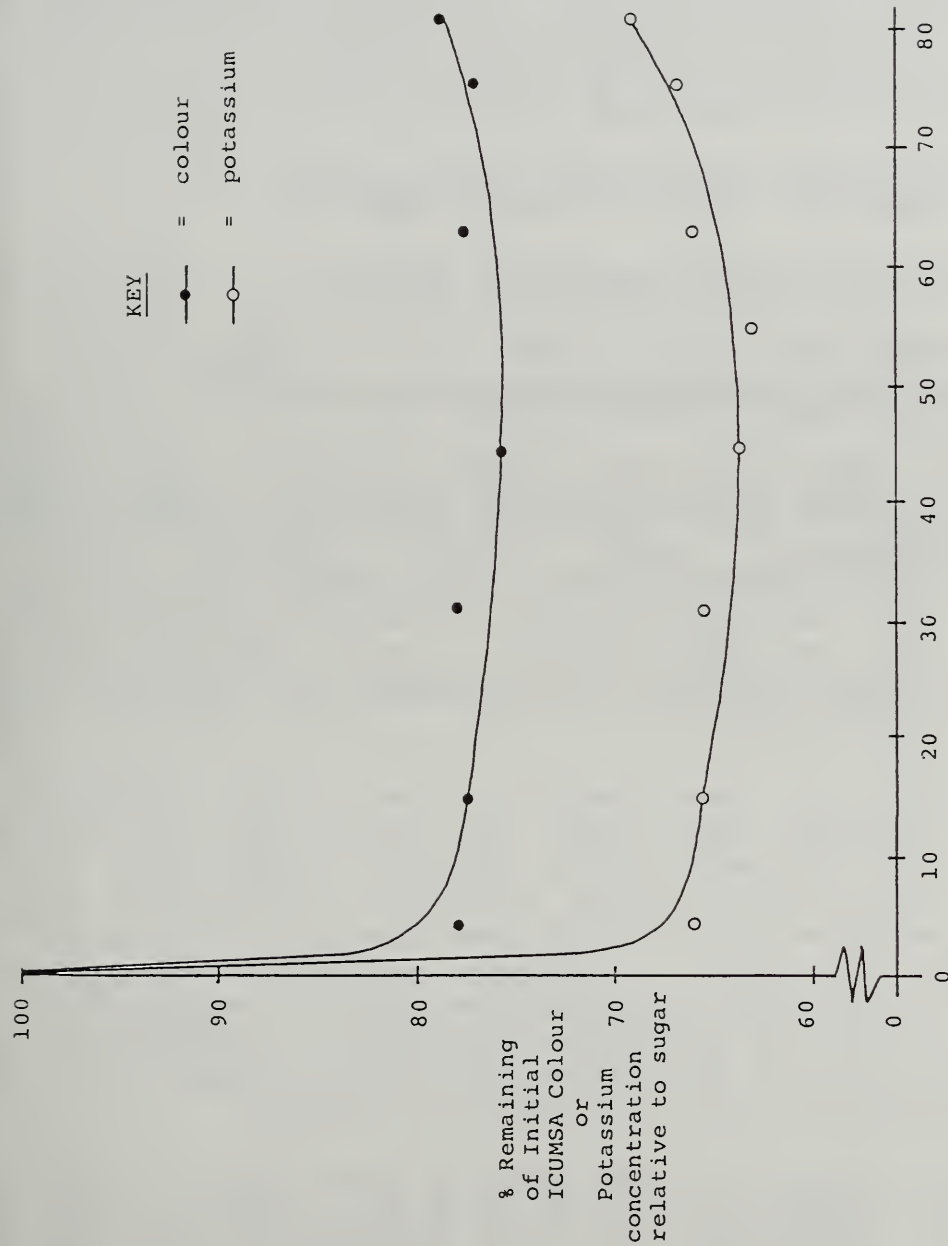


Figure 9 Colour and potassium distribution in production white sugar

the "external" colour has been calculated, the difference between this and the measured solution colour of the sugar provides an estimate of the "internal" colour. As our work indicates that much of the potassium is "internal", the "external" colour so calculated is over-estimated, i.e. is a maximum; the "internal" colour estimate is correspondingly a minimum.

Elimination factors were calculated as the ratio between the potassium or colour (relative to sugar) in the white massecuite and that in the white sugar. Thus:

Potassium elimination factor =

$$\frac{\text{Potassium (mg/kg sugar) in massecuite}}{\text{Potassium (mg/kg) in white sugar}}$$

Solution colour elimination factor =

$$\frac{\text{Massecuite solution colour (I.U.)} \times \frac{100}{\text{Purity}}}{\text{White sugar solution colour (I.U.)}}$$

The data in Table 7 show the results for seven factories for sugars produced either during the 1984/85 beet campaign or during subsequent thick juice refining runs. The sugars examined had solution colours ranging from 13.4 to 23.3 I.U. The calculated minimum "internal" colour ranged from 7.8 (48% of total colour) to 14.8 (64%) and the calculated maximum "external" colour ranged from 5.2 (29%) to 11.0 (56%) ICUMSA units.

The significance of these results relates to the technical means of producing white sugar with both colour and ash specifications. If much of the solution colour exists as "internal" colour, then normal centrifugal washing will not reduce the solution colour to the same extent as it does the ash content, if this is largely "external". In such circumstances, the most cost-effective means of meeting both specifications may be the adoption of methods for separately regulating the ash and the colour; washing to regulate colour in such circumstances may be an expensive solution.

On average, a minimum of 57% of the solution colour of the white sugars studied was calculated to be "internal". This finding is consistent with the hypothesis from our previously-reported work (Shore et al. 1984) that it is high molecular weight colorants within the crystal that are mainly responsible for white sugar colour.

Since a significant proportion of the potassium is present inside the crystal (possibly associated with mother liquor inclusions), some of the calculated "external" colour may

Table 7 Average colour and potassium contents of factory white massecuites and white sugars
(Data for 7 factories, 1984/85)

Factory	White Massecuite		White Sugar		Elimination Factor		White Sugar Solution Colour		
	Solution colour (I.U. on sugar)	Potassium (mg/kg on sugar)	Solution colour (I.U. on sugar)	Potassium (mg/kg on sugar)	Solution colour	Potassium	Calculated minimum "Internal"	Calculated maximum "External"	Internal as % of total colour
A	2167	8759	18.1	33	120	265	9.9	8.2	55
B	1999	8411	17.7	22	113	382	12.5	5.2	71
B-JR	2370	7532	21.1	31	112	245	11.4	9.7	54
C	1857	8498	13.4	25	139	340	7.9	5.5	59
D	1792	8254	16.1	38	111	217	7.8	8.3	48
D	2265	8516	23.3	32	97	266	14.8	8.5	64
E	1971	7461	16.7	21	118	355	11.2	5.5	57
E-JR	2241	7349	16.8	21	133	350	10.4	6.4	62
G	2760	6675	23.2	26	119	257	12.5	10.7	54
G-JR	2446	6673	19.5	30	125	222	8.5	11.0	44
H-JR	2536	8605	17.0	28	149	307	8.7	8.3	51
Average	2219	7885	18.4	28	121	291	10.5	7.9	57

JR = Thick juice refining run

really be colour associated with syrup droplet inclusions within the crystal. As yet we have no proof of such a phenomenon. As an added complication, it has been demonstrated (Guo and White 1984) that, rather than droplet composition being similar to that of the mother liquor, colour can be concentrated within inclusions, by a factor of about four times with respect to inorganics such as potassium.

In summary, the colour of white sugar can be considered as comprising an "external" colour associated with the outer layers of the crystal and removable by washing, and an "internal" colour which may be a combination of adsorbed colour and colour included within droplets of impure syrup. This "internal" colour can be regarded as 'irreducible', in the sense that it is not progressively decreased by continued washing.

This theory supports our previous observations (Shore et al. 1984) of high, medium and low molecular weight colorants inside the crystal but only medium to low molecular weight colorants on the surface. If colour selectively adsorbed by the crystal is of high molecular weight this becomes concentrated inside the crystal relative to the other, lower molecular weight colorants. The surface colour comprises predominantly the lower molecular weight colorants as does the mother liquor.

3.4.4 Use of colour to ash ratios

On occasion, factories may need to use additional centrifugal wash water to maintain the white sugar solution colour at the required target value. In consequence, there is a low final concentration of the non-sugars on the surface of the crystals, resulting in a low ash sugar. Factories with difficulties in regulating white sugar colour often produce a normal coloured sugar but with an ash content significantly lower than normal as a consequence of increased wash. This situation is characterised by a high elimination factor for potassium. From Table 7, factories B and C in campaign and factory E in both campaign and thick juice refining run are seen to fall into this category.

Extending these considerations, the ratio of solution colour to ash for white sugars, rather than the colour alone, is an important tool in determining how serious a colour problem is at a factory. By taking the ash content of the sugar into account, some allowance is made for the effect on sugar colour of varying wash water applications.

Analysis of unpublished factory data has shown that, for British Sugar granulated white sugars, values in excess of 2000 for the ratio of colour to conductivity ash are higher

than average. The value of 2000 represents a sugar of 20 ICUMSA units solutions colour and 0.010% ash, the latter value being the maximum target value within British Sugar. Where no special colour control difficulties exist, washing is adjusted to achieve an ash value close to the target but appreciable extra water may be used in an attempt to reduce white sugar colour where this is higher than normal. This is reflected in the analytical data, which show that the ash content of sugars with colour to ash ratios in excess of 2000 are usually markedly below 0.010%. As the colour to ash ratio increases, the economics of using washing to regulate colour become more adverse and the difficulties are increased if a significant amount of "internal" colour is present.

4. DISCUSSION

The colour balance described in this paper utilises basic factory information and, from this, it is possible to establish the major sources of colour in the process at each factory. Of particular importance, it is possible to calculate the relative proportions of the colour in the sugar-end products which were made by chemical reaction in the sugar end or which have their origins in thick juice. This approach will have research and technical applications, both for identifying areas of poor colour control in the factory and for monitoring any effect of changes in process conditions upon the colour distribution in the factory.

A second tool described in this paper is the ratio in white sugar of colour to ash, as an indicator of colour problems at a factory. Any excessive washing to produce sugar of an acceptable colour would result in low ash and hence a high ratio of colour/ash.

The work on elimination of colour and potassium during the boiling of white sugar has facilitated calculations to show that, on average, a minimum of about 60% of white sugar colour is present in the crystal itself. From our previous work (Shore et al. 1984) we hypothesised that high molecular weight colorants from the sugar end may be responsible for much of the colour of white sugar. We have now found that most of the colour of standard liquor, from which white sugar is crystallised after boiling, originates in the beet end.

These three observations give rise to two main possibilities for controlling white sugar colour: either to regulate beet-end colour in some way so that, in the sugar end, there is less formation of the high molecular weight compounds or to regulate these high molecular weight compounds in the sugar end before they are returned to standard liquor. From our future research programme, we shall learn which option is preferable. This will entail consideration of the relative

importances of operating on the overall colour, on particular juices or on specific compounds in order to obtain the optimum improvement in white sugar colour.

The work reported here highlights the importance of a high elimination of colour from the white sugar during crystallisation. A good separation of syrup from crystals is also needed, necessitating minimum conglomerates, minimum small crystals and no syrup inclusions, so that there are maximum efficiencies both of separation in the centrifuge and of washing.

However, as the work shows, even normal-coloured syrup can contain colorants which become built into the sugar crystal. There is little the factory can do about this once such colorants are present in the syrup unless a colour removal plant is available. The chemical nature of these colorants (reviewed by Shore et al. 1984) suggest that their formation can be restricted in the process by keeping invert sugar concentrations low and by avoiding long heating and retention times in evaporators and heaters. The deterioration of beet towards the end of the campaign, with accompanying increase in invert sugar content, should likewise be guarded against to limit the potential for formation of such colorants. There are, of course, other good reasons for wishing to minimise invert sugar production and destruction in beets and in the process.

In order to develop optimal technical methods of controlling white sugar colour, it is essential to know more about the nature of the colorants giving rise to the majority of the sugar colour, about their location on or in the crystal and about the mechanisms for their location.

5. CONCLUSIONS

1. Gel permeation chromatographic studies of colorants separated from juices and beet sugars from British Sugar's factories indicate that relatively high molecular weight colorants (1,000 - 5,000 daltons) are incorporated into the white sugar crystal while medium to low molecular weight (up to 1,000 daltons) colorants are found both inside and on the surface of the crystal.

2. The majority of the colour of white beet sugar is apparently distributed evenly through the majority of the crystal. On average, the colour of the sugar can be reduced by 25% by dissolving 20% of the crystal but further removal of sugar produces no improvement in colour. This applies to both high and low colour sugars, i.e. the 'extra' colour in a high colour sugar is not located specifically in the surface layers but is distributed, apparently evenly, through the crystal. It would thus seem that high molecular weight colorants may be

responsible for most of the colour of white beet sugar.

3. The colour balance technique enables the pattern of colour movement and colour formation in the process to be identified.

4. For British Sugar's processing conditions, relatively large colour increases occur during evaporation and in the after-product sugar boiling and crystallisation stages. The colour increases occurring during the white and raw sugar boilings are relatively smaller.

5. On average, only 7% of the colour of standard liquor is synthesised in or after the white pans. A further 13% of the colour is formed in the dissolving system during the production of standard liquor. The remaining 80% is thick juice colour either passing forwards directly to the standard liquor or being recycled to the standard liquor as part of the sugar-end return colour. This thick juice colour originates from that present in thin juice (62%) and that formed during evaporation (18%).

6. Colour elimination factors can be used to predict when action may need to be taken if white sugar colour is not to exceed the target. This could form the basis of computer control of colour in a factory.

7. On average, the elimination of colour between white massecuite and white sugar is about 120-fold, whereas that of potassium is 290-fold. Colour is thus less likely to be eliminated from the crystal than potassium; presumably, the determining mechanisms are different for colour and ash.

8. The ratio of colour to conductivity ash for granulated white sugar may be used as an indication of colour problems at a factory. Values in excess of 2000 in British Sugar are higher than average and are usually associated with sugars of normal colour but low ash, suggesting excessive use of centrifugal wash water to regulate colour.

9. By calculation, an average of 40% of white sugar colour is associated with the film of residual syrup around the crystal. 60% is incorporated in the crystal as it grows in the vacuum pan; the mechanism may be one of droplet inclusion or of uniform adsorption.

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DISCUSSION

C. C. Chou, Amstar - Is the color balance technique in use in your factory used for troubleshooting purposes?

Broughton - The factories have the equipment now to do the ICUMSA color measurement and have been doing so for about a year. They have the computers to carry out the balance because it can be very tedious. For the present, because it is an experimental technique in the factory, the research laboratories themselves are supplying the people to actually do the work. At the moment, we are just looking for areas where we can easily detect large color rises so that we can concentrate our efforts in those areas. It might be a process that we could computerize entirely so that it was done automatically by the factory, but it is not being done at present by the factory staff.

Chou - Thank you. Have you considered classifying the colorant based on absorption wavelength? If we look at your first figure, most of the absorption peaks are below the visible spectrum, which ranges from 375 nm to 750 nm. Most of those are in the UV.

Broughton - That is true, you certainly can't see down in that range. The only problem with doing what you suggest is the very small amount of colorant present. It is not possible to get absorbance in the visible region from the small amount that is extracted from different parts of the crystal. So you have to use a more sensitive technique. Some of these molecules have very large absorbances in the UV so they provide a method of finding out what is there, but perhaps do not give a very clear idea of what is causing the color.

Chou - Would you consider this group as color precursors?

Broughton - Some of them may be color precursors, but we have not gotten as far as that yet.

Stanley Bichsel, American Crystal Sugar - You indicated in your paper that the tyrosine oxidation reaction was perhaps limited at British Sugar due to the type of diffuser that you use and also chemical treatment. Could you elaborate on that?

Broughton - We have 3 diffuser types. We have the DDS diffuser, which is very recent to us, we have the RT and tower type diffusers, and we have most of our experience with the RT and tower types. We have very limited experience with the DDS type.

We have carried out work to measure the oxygen concentration in the diffusers, and it is quite low in all these types of diffusers. Nevertheless, we do consider it is certainly higher in the RT type of diffuser, which is probably as you would expect. Some authors say that the oxidation of polyphenols is characterized by a grey appearance of second carb juices. The only times we have experienced this is in the tower diffuser which has the lowest juice oxygen content. We believe that by virtue of the mixing action of the different types of diffusers, there is just enough

difference in oxygen content to cause the oxidative reactions to proceed before carbonatation so that the melanins are then removed on the precipitate rather than going through into second carbonatation where they will be oxidized and give us great color, which then passes through the process.

Bichsel - I recall some time back a paper relating the invert content of thin juice and the usage of sulfur dioxide. I believe it was a paper from British Sugar by Carruthers, Shore and Oldfield. Have you noticed in your work a relationship between invert levels and sulfur dioxide usage as far as color prevention?

Broughton - There is not any doubt that invert sugar is one of the causes of color in the juice stream. One of the great difficulties we have is persuading our factory management that the color of the juice stream is not necessarily indicative of the white sugar color. We know, as many of you know, that it is possible to boil a very good white sugar out of a very dark juice at times. Certainly we know that invert level contributes to juice color. We know that sulfur dioxide plays an important part in regulating that color. What we cannot say with certainty is whether those things contribute to the white sugar color in any significant way.

Andrew VanHook, Holy Cross College - Have you noted any segregation of the color within an individual crystal? In our own work with inclusions, when we have used syrups tagged with things such as fluorescein, we note that the inclusions are more predominantly at the right prism end of the crystal than in other sections of the crystal.

Broughton - We have not made studies on individual crystals in any of the detail some of our colleagues in other companies have done. In this respect we have worked closely with Professor Mantovani in Ferrara and also with Pieter Van der Pol at Central Suiker in the Netherlands. They have made many more studies on this than we have, and I am not aware from their work if they have observed inclusions predominantly in any particular face of the crystals.

FLAVORS FROM BEET AND CANE SUGAR PRODUCTS

Mary An Godshall

Sugar Processing Research, Inc.

INTRODUCTION

The development of flavor in sugar products is a subject of interest to the sugar producer as well as the end user, be it a food processor, beverage manufacturer, or home consumer. The flavor of a sugar product results from the nature of the starting material, i.e., cane, beet, sorghum, or corn, and is modified to its final form by its processing history. Flavor is usually a fortuitous result of the process, producing the proper array of desirable constituents and odors. Not much attention needs to be given, therefore, to the flavor, once the process is in place, until a problem with an off-flavor arises or a customer specifies a particular attribute.

In the following paper, several topics related to flavor in beet and cane products are discussed. These include the evaluation of several methods for obtaining volatile components from molasses, the examination of various flavor fractions from beet molasses and other products, and the effect of adsorbent treatment on organoleptic properties. Several flavor problems encountered by the sugar industry will be highlighted.

Molasses made from beet sugar has a set of distinctive, characteristic odors that immediately distinguishes it from molasses made from cane sugar. These odors are complex and include hydrolyzed protein, meaty, earthy, amine and possibly some sulfurous notes. Since these odors are not the browned-sweet-caramel odors commonly associated with sweet products, beet molasses is not suitable for use as a food or flavoring without additional treatment. However, the Japanese have recently described the use of beet molasses to derive a "seasoning" in which burnt-sugar and brown-caramel notes were considered unacceptable (Kumada et al. 1985). Presumably this product was to be used in a manner similar to soy sauce seasoning.

Numerous compounds have been identified in beets. Volatile components from young and mature sugar beet leaves were compared (MacLeod et al. 1981). Twenty-nine compounds were identified and another 7 were partially characterized. Tressl et al. (1976) identified over 60 volatile components in molasses. An exhaustive study of pulp drier volatiles (Oldfield et al. 1980a and 1980b) identified numerous compounds, with the conclusion that no single compound was responsible for the characteristic odor, which was

probably produced by a combination of many contributing compounds. These studies point up the complexity of the odor of beet products, which consists of many highly aromatic (odorous) constituents, such as pyrazines, pyrroles, pyridines, furans, phenolics, and volatile acids.

Treatment with various adsorbents was examined as a means of removing objectionable components. The adsorbents used in this study are listed in Table 1. Most of them are already in use in the food industry and have many useful applications in food processing and purification.

Recent publications reflect the continued interest in adsorption treatment. Florisil was used to reduce bitterness and tartness in grapefruit juice (Barmore et al. 1986). Alumina selectively retained hop bittering components (Lam et al. 1986). Activated carbon and ion exchange (cationic followed by anionic) were evaluated for removal of phenolic compounds from soy protein extracts (How and Morr 1982). Both treatments resulted in improved flavor and odor but no reduction in bitter and astringent intensities, based on sensory analysis. Cation exchange resin, PVP, and XAD-4 were evaluated for improvement of pear juice storage (Cornwell and Wrolstad 1981). Bentonite is used to bleach and deodorize edible oils (Delaney 1986).

MATERIALS AND METHODS

Extraction of beet molasses. Extraction of beet raw sugars and beet molasses with methylene chloride (CH_2Cl_2) into basic, neutral, acidic, and phenolic fractions was accomplished by the following manner: 50 g molasses diluted with 150 ml H_2O (1:3 wt/vol) and 6 g Na_2CO_3 added (pH about 10.5). Extracted with three 100 ml portions CH_2Cl_2 . This fraction contained basic and neutral compounds. The extract was concentrated to about 50 ml and extracted with three 20 ml portions of 5% HCl . The remaining CH_2Cl_2 contained the neutral fraction. The aqueous fraction was adjusted to pH 10.4 with NaOH and extracted with ether. This contained the basic fraction. The original (previously extracted) molasses was adjusted to pH 2.3 with 4N H_2SO_4 and extracted with three 100 ml portions of CH_2Cl_2 to give a fraction containing acids and phenolics. The extract was washed three times with 20 ml portions of 5% NaHCO_3 . The phenolic fraction remained in the CH_2Cl_2 . The aqueous bicarbonate solution was acidified to pH 1 with 10% HCl and extracted with ethyl acetate to give a fraction rich in acids.

The fractionation scheme is diagrammed in Figure 1.

Other extraction procedures. Continuous liquid-liquid extraction with CH_2Cl_2 was done using similar solutions and fractionation described above. Solutions were extracted for 3 hours; extracting solvent was replaced with fresh solvent, and extraction continued for another 3 hours.

TABLE 1.--Characteristics of adsorbents used to treat beet and cane molasses for flavor and odor improvement

Adsorbent	Description	Applications
Bentonite	Bleaching clay; colloidal hydrated Al silicate	Adsorptive bleaching of edible oils; affinity for carotene, chlorophyll, phospholipids, metal oxides, oxidation products of triglycerides; immobilization of dextranase.
Carbon	Granular activated form	Removes phenolics from soy protein to improve flavor; sugar processing; many other uses.
Alumina	Al_2O_3	Adsorption of bitter compounds from hops; adsorbs catechol function.
IRA 118H	Sulfonic acid polystyrenic (strong) acid ion exchanger	Inversion of sucrose.
IRA 958	Strong base acrylic ion exchanger, quaternary ammonium function	Removes color and organics from sugar solutions.
PVP	Polyvinylpyrrolidone (Polyclar-AT)	Removes phenolics from pear juice; tannins in beer and wine; prevents haze in beer and wine.
XAD-2	Low polarity styrene-DVB copolymer	Many research applications; not yet approved for food usage.
Florisil	Activated Mg silicate	Reduction of citric acid, limonin, and naringin in grapefruit juice to lessen bitterness.

Soxhlet extraction of 100 g raw beet sugars was carried out for 2-4 hours. Soxhlet extraction of 50 g beet molasses mixed to a flowable powder with 30 g silica gel was also attempted, but this failed to extract the characteristic beet odors, giving only floral and caramel compounds.

A dry column extract of raw sugar was performed in the following manner: 100 g sugar, granular or ground to a powder in a mortar, was packed into a 330 mm x 22 mm chromatographic column (Kontes Scientific Glassware) (Lam et al. 1986). Methylene chloride was added to fill the column reservoir and left in contact with the sugar for 5 min and removed with gentle suction. This was repeated once with fresh solvent.

All extracts were dried over Na_2SO_4 and filtered on a sintered glass funnel prior to concentration for gas chromatography. Odor of extracts was determined by placing a drop of concentrated extract on a strip of filter paper and allowing a few seconds for the solvent to evaporate. The odor was noted over a period of time since the character of the extracts changed as volatile compounds evaporated.

Volatile profiles. Headspace volatiles were stripped from molasses, trapped on Tenax-GC, and thermally desorbed onto a fused capillary column using the External Closed Inlet Device (ECID) (Scientific Instrument Service, River Ridge, LA). Volatiles were stripped from 10 ml molasses, diluted 1:1 (wt/wt) with water, for 30 min using nitrogen at 120 ml/min; the Tenax cartridge was at ambient temperature; the molasses solution was maintained at 80° C during the stripping period. A 125 ml gas washing bottle was used as the sparging vessel for obtaining volatiles. The apparatus is diagrammed in Figure 2.

Gas chromatography. Extracts were analyzed on a 30 m fused silica column coated with DB-5 (cross-linked and bonded 5% diphenyl polysiloxane), 0.32 mm I.D., 1 μ film thickness, using a 1:30 split. The program used was: 100° for 4 min; 4°/min to 265° for 10 min.

For the analysis of volatiles trapped on Tenax, the volatiles were desorbed from the Tenax for 3 min at 185° onto the column maintained at 0° for 3 min. The temperature was raised 10°/min to 50°, held for 1 min and then raised 4°/min to 200° and held for 10 min.

Mass spectroscopy. Mass spectroscopy was performed on a Finnigan 4500 GC/MS. The operating parameters were 70 ev ionizing potential with scan range of 33-450 amu/s. Fused silica columns were used, 38 m or 50 m, coated with cross-linked SE54, 0.3 mm I.D., 0.5 μ film thickness. Volatiles were desorbed from the Tenax for 3 min onto the column at -30°. The column temperature was programmed 15°/min to 30° and then 4°/min to 250° for 5 min.

Adsorbent tests. Adsorbents were washed with water, fines decanted and filtered on No.4 filter paper to remove excess water and used wet. The 958 resin was regenerated with 10% NaCl and

- 1 Nitrogen tank
- 2 Pressure regulator
- 3 Flow valve
- 4 Stainless steel line
- 5 Hydrocarbon trap
- 6 Gas washing bottle
- 7 Heating tape
- 8 Sample
- 9 Tygon sleeve
- 10 Tenax trap
- 11 Rheostat

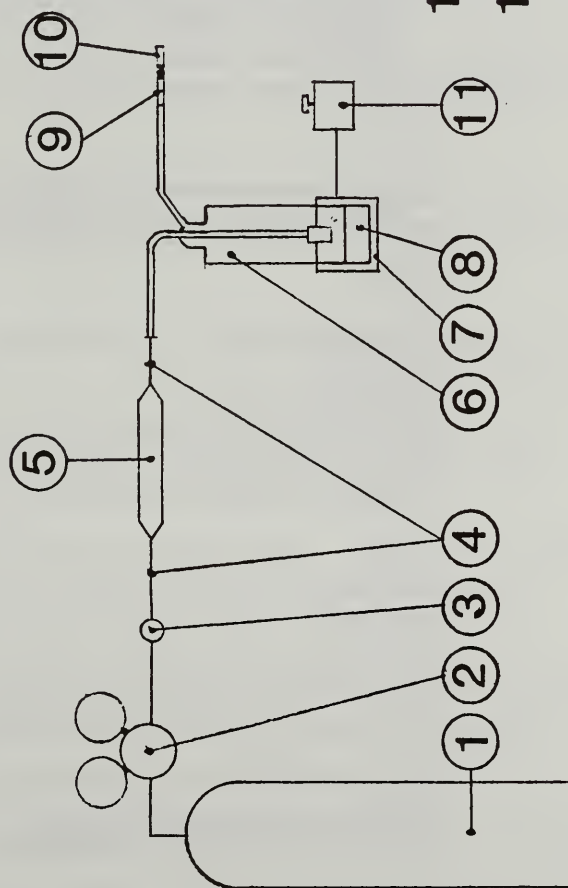


Figure 2. Apparatus used to purge and trap volatiles from molasses.

washed to remove chloride ion. The 118H resin was regenerated either with 10% NaCl or 1% HCl. All resins were thoroughly rinsed immediately prior to use. Treatment consisted of shaking 50 ml of 10% (wt/wt) molasses with 1 g adsorbent for 30 min. Solutions were filtered on No. 4 paper to remove adsorbents. Treated solutions were analyzed for odor, brix, pH, phenol, amino N, 420 color, and UV absorbance. Cane molasses was also treated and analyzed for odor, flavor, phenol, and amino nitrogen. The Folin-Ciocalteu reagent was used for analysis of phenols (Godshall and Roberts 1983) and the Moore and Stein (1954) ninhydrin method for amino nitrogen.

RESULTS AND DISCUSSION

Comparison of extraction methods. The liquid-liquid extractions of beet molasses, both manual and continuous, gave extremely complex chromatograms. Figure 3 shows the manually extracted basic-neutral fraction (B extract), which contained more than 250 peaks. Figure 4 shows the continuous extraction of the acids-phenolics fraction (A extract), also having more than 200 peaks. Manual and continuous methods gave comparable results as long as the loss of volatiles was minimized by using subambient condenser water during continuous extraction. Manual extraction had the advantage of being quicker but was plagued by the formation of nearly intractable emulsions. These were broken by the addition of Na_2SO_4 .

When the extracts were subdivided into basic, neutral acidic, and phenolic fractions, the chromatograms were considerably simplified.

The odors of the various fractions were as follows: (Numbers in parentheses refer to Figure 1.)

B extract: Strong characteristic processed beet odor; strong nutty; slight fruity and caramel.

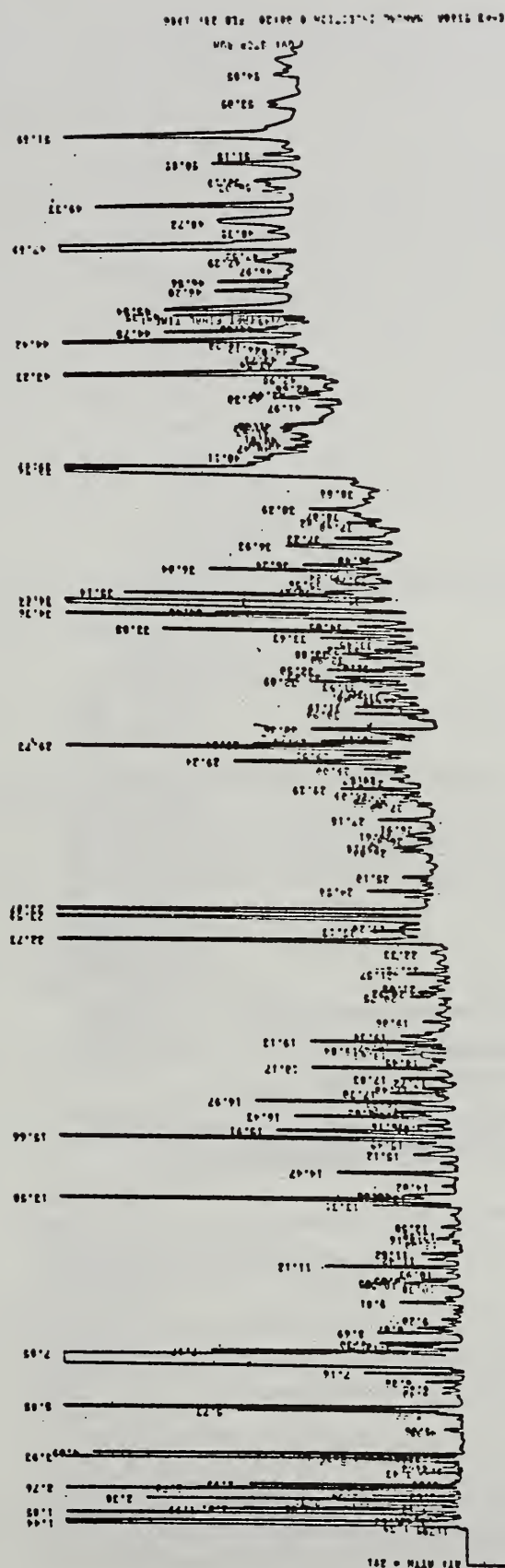
Neutral fraction (B-1): Many odors with unpleasant notes predominating; first noted are burnt-bone odors and processed beet; next are some caramel notes.

Basic fraction (B-2): Very strong nutty but also some pyridine type odors similar to collidine and lutidine; some faint floral.

A extract: Acid, pungent, butyric; strong caramel, fruity.

Phenolic fraction (A-1): Caramel, burnt sugar, sweet-browned odors; very pleasant.

Acid fraction (A-2): Sharp, pungent, valeric, butyric; underlying these are floral, fruity, and caramel odors.



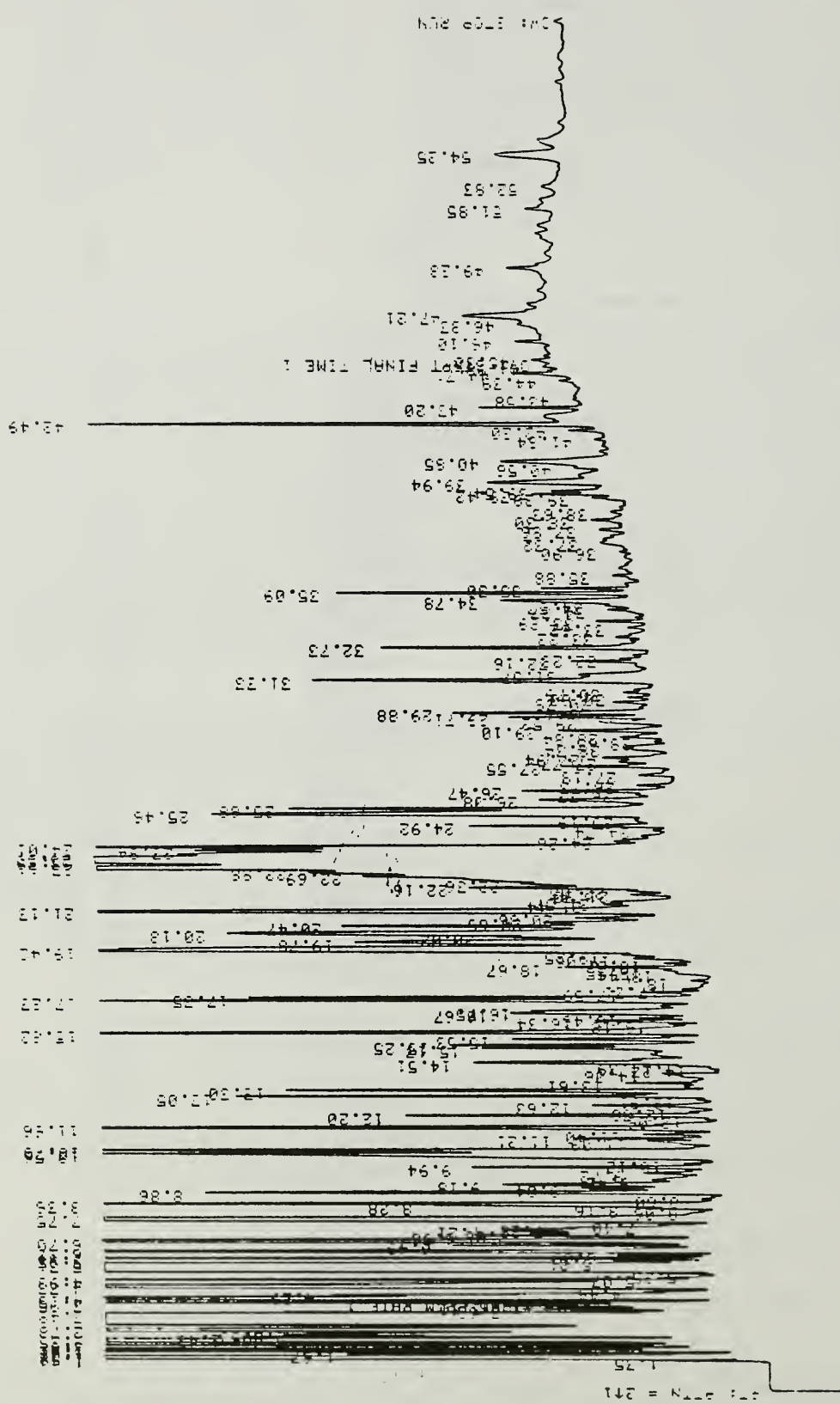


Figure 4. Acid methylene chloride extract of beet molasses after basic extraction. GLC conditions are described in text.

INJECTION 1 14:05 APR 7 1986

It was of interest to note that when an attempt was made to extract molasses in a Soxhlet extractor by mixing the molasses to a flowable material with silica gel, the only compounds extracted were those responsible for floral and caramel odors.

Extraction of raw beet sugar. Liquid-liquid extraction of raw and refined beet sugars with a characteristic off-odor did not produce an extract with the odor when CH_2Cl_2 was the extracting solvent. Soxhlet and dry-column extracts were more promising. Figure 5 compares the two methods of extraction on a raw sugar. Both methods extracted similar peaks but their proportions were quite different. The major peak in the dry-column extract, with retention of 7.47 min, was identified as heptanoic acid; this was also a major peak in the molasses extracts, and while it would not be responsible for the off-odor, imparting a mild fatty odor instead, it may act as a marker for a problem sugar.

Odor of Soxhlet extract: Acid, valeric, some floral, sweaty socks; later fatty and nutty. Some characteristic odor remained in the extracted sugar.

Odor of column extract: Acid, valeric acid, doggy; later fatty. Some odor also remained in the extracted sugar.

Headspace volatiles. Volatiles stripped from 10 ml 50% molasses are shown in Figure 6. This method was reproducible to within 5-6% as long as conditions were standardized, and may be used to profile molasses and the effect of various treatments.

Identification of compounds. Because of the complexity of the extracts, many peaks remain to be identified.

Table 2 lists the compounds identified by GC/MS in a basic CH_2Cl_2 extract of beet molasses, beet molasses volatiles trapped on Tenax, and volatiles vented from a steam distillation of molasses. The latter was of interest because of a distinct chocolate-aroma that was noted exiting the vent during the distillation.

Many of the volatile constituents identified in Table 2 have strong distinct odors, such as the pyrazines, sulfides, thiazoles, and substituted pyridines. These contribute to the aroma of many foods, particularly meats, coffee, and chocolate. Dimethylsulfide, found in trace quantities in this beet molasses, is a characteristic odor-impact compound in cane molasses (Godshall et al, 1980).

Treatment with adsorbents. Table 3 summarizes the results of adsorbent treatment of beet molasses, and Table 4 lists the odors that resulted. The molasses had two peaks in the UV range, a distinct peak of maximum 261.5 nm and a shoulder at about 316.5 nm, and the effect on these components was measured as well. Figure 7 shows the UV spectra of treated molasses. Brix was not affected by any adsorbent. The pH of 8.28 remained unchanged, except for 118H (NaCl) where it dropped to 7.10 and 118H (HCl) where it dropped to 5.75.

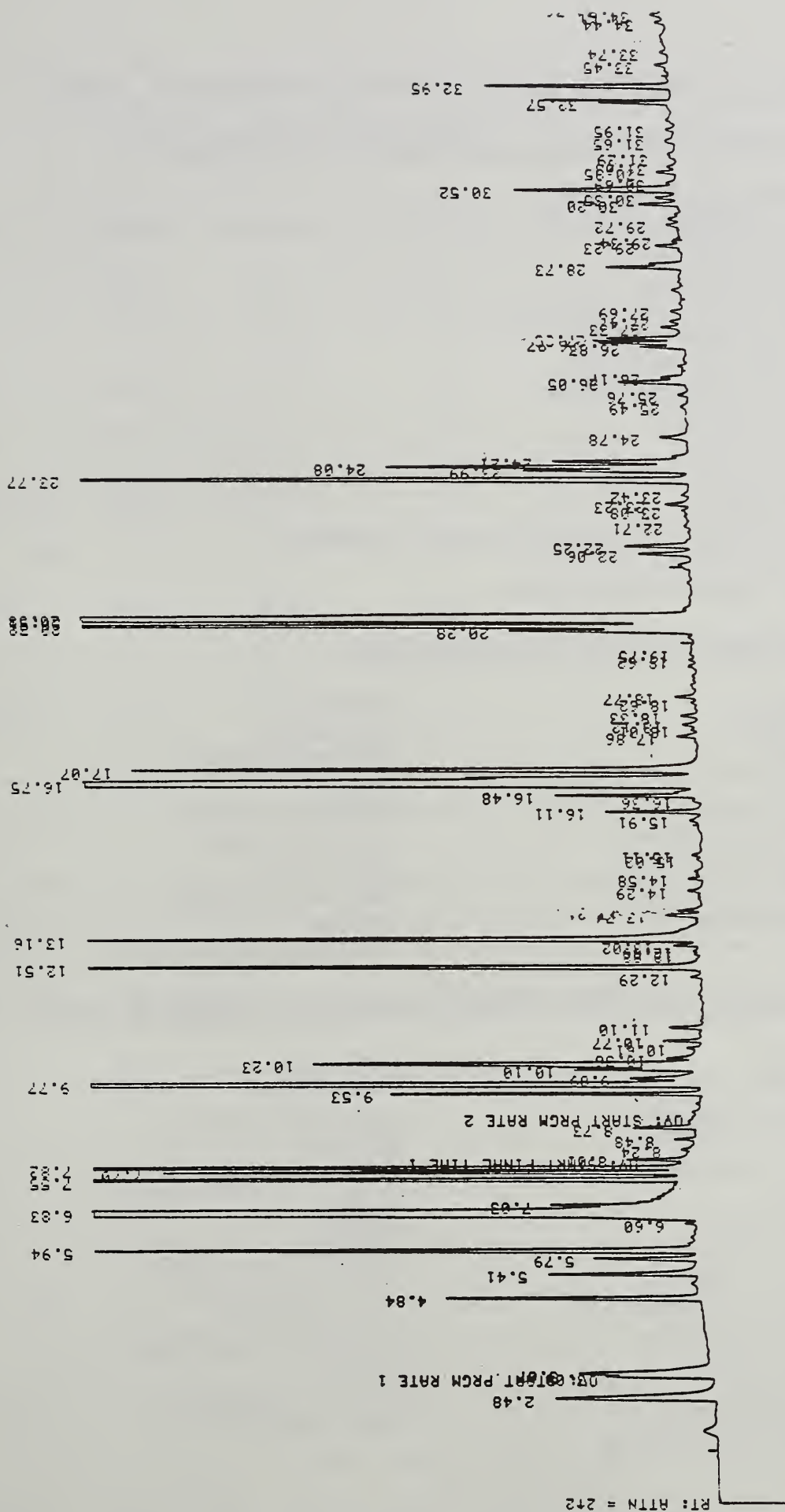


Figure 6. Beet molasses volatiles trapped on Tenax-GC. GLC conditions described in text.

TABLE 2.—Compounds identified in beet molasses by GC/MS

Compounds identified in basic extract of molasses

2,5-Dimethylpyrazine
 Trimethylpyrazine
 2-Pyrrolidinone
 2-Piperidinone
 4-Methyl-5-thiazolethanol
 1-Butyl-pyrrolidione
 2,6-Dimethoxyphenol
 3-Pyridinecarboxamide
 Heptanoic acid
 Heptadecane
 Tetradecanoic acid
 Octadecane
 2,5-Dimethyl-3-(2-methylpropyl)-pyrazine
 9,12-Octadecadiene-1-ol
 9,12-Octadecadienoic acid

Headspace volatiles trapped on Tenax

Ethanol	Diacetyl
Acetone	2-Butanone
Butane	2-Methyl-butanal
Furan	Benzene
Acetaldehyde	3-Methyl-butanal
Dimethylsulfide	4-Penten-2-one
Carbon disulfide	2-Pentanone
2-Methyl-propanal	Dimethyldisulfide
Propanenitrile	Toluene
Acetic acid	2,5-Dimethylpyrazine

Volatiles vented from steam distillation, trapped on Tenax

Pentane	Pentanal
Acetone	2-Pentanone
Dimethylsulfide	3-Methyl-1-butanol
Carbon disulfide	Heptane
Methyl propanal	Dimethyl disulfide
Hexane	Toluene
2-Butanone	Hexanal
Ethyl acetate	k-Methyl-isothiazole
Methyl furan	2-Methyl-pyridine
3-Methyl-butanal	Butyl acetate
Methyl pyrazine	2-Methyl-3-heptanone
2-Heptanone	Xylene
Heptanal	Dimethyl pyrazine
2,3-Octanedione	Benzaldehyde
Dimethyltrisulfide	

TABLE 3.-- Summary of adsorbent treatment of beet molasses

Adsorbent	<u>Percent removal of component</u>					Improvement in odor
	Phenol	Amino N	261.5nm	316.5nm	420nm	
XAD-2	24.5	34.6	14.6	15.1	12.8	None
PVP	32.7	27.7	8.2	8.1	5.9	None
Alumina	22.7	0	6.4	10.2	11.7	None
Florisil	30.3	0	6.0	9.2	8.2	None
Bentonite-NG	21.4	15.6	8.4	11.5	9.6	Slight
Bentonite-160	17.1	25.9	6.5	8.5	7.2	None
958 (NaCl)	35.9	38.7	32.1	44.4	59.0	Slight
118H (HCl)	17.8	62.4	11.4	4.9	13.2	Moderate
118H (NaCl)	19.6	0	7.5	4.4	7.8	Slight
Carbon	39.1	55.1	41.4	28.4	21.8	No odor left

TABLE 4.--Odor of beet molasses before and after adsorbent treatment

Adsorbent	Odor of molasses
No treatment	Characteristic strong processed beet odor; slightly amine and ammoniacal.
XAD-2	High amine notes gone; recognizable beet odor; unpleasant.
PVP	Slightly acidic; lower beet odor; unpleasant.
Alumina	Beet; meaty.
Florisil	Similar to untreated sample.
Bentonite-NG	Faint beet; slightly floral; slightly dusty.
Bentonite-160	Dusty (from clay); beet.
958 (NaCl)	Mild beet.
118H (HCl)	Acidic and floral; faint burnt sugar.
118H (NaCl)	Mild beet; floral notes; faint burnt sugar.
Carbon	Almost no odor.

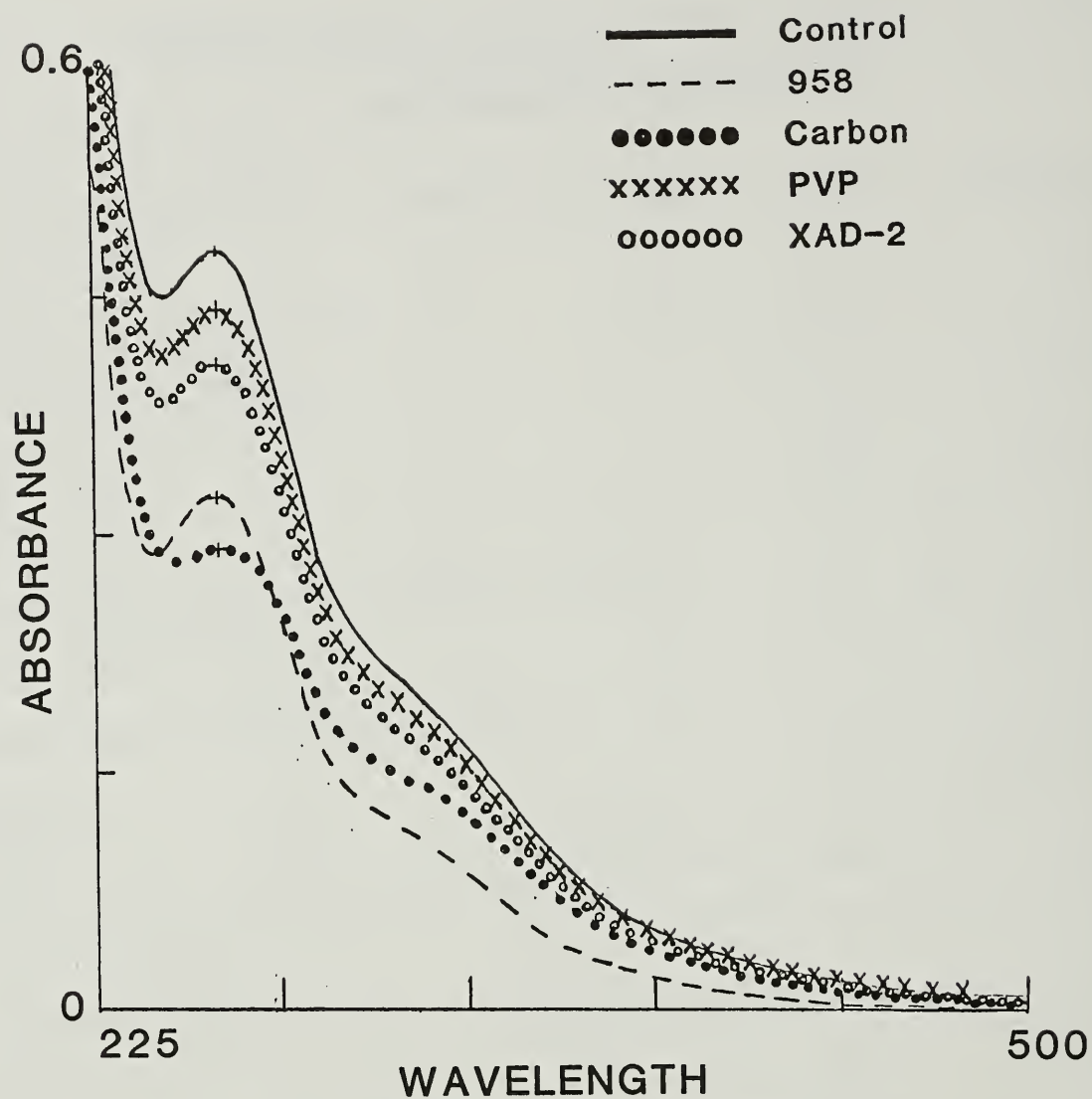


Figure 7. UV scan of beet molasses before and after treatment with adsorbents.

Table 5 summarizes the results of adsorbent treatment on a sample of cane molasses.

Comparing the effect of the adsorbents on the flavor/odor of cane and beet molasses emphasizes the difference in the composition of flavor compounds in the two products. While cane molasses is quite low in base-extractable compounds, it does have the tendency to develop extremely harsh and bitter flavors. Adsorbents such as XAD-2, PVP, and carbon are able to moderate these attributes and improve flavor. Cationic resin in either the H^+ or Na^+ form helped improve the bitterness but emphasized the acidity. The anionic resin removed the acidic and fruity compounds, producing an unacceptable product.

Whereas XAD-2 and PVP helped improve cane molasses flavor, they had no effect on beet molasses, and the best adsorbents for odor improvement of beet molasses were cation exchanger in the H^+ form and activated carbon.

CONCLUSIONS

It is not yet possible to name the individual compound or compounds responsible for the characteristic odors of beet products, and it may result that the odor is due to many compounds (Oldfield et al. 1980a). Certain highly complex foods, such as chocolate and coffee, that have undergone a lot of processing, are characterized by hundreds of compounds, no single one of which is responsible for their odors.

An important factor in reducing beet-derived odors appears to be adjustment of pH. Lowering the pH to 6.5 or below markedly suppresses the odor. However, care must be taken in using this approach, since these types of compounds, while no longer imparting a flavor or odor at low pH, can cause a beverage (i.e., non-cola or fruit) to taste bland or flat, probably due to these basic compounds complexing with the acidic flavor function. Many flavors especially fruit types are slightly acidic due to carbonyl and ketone functionalities and can easily react to lose their flavors.

The neutral and basic fractions contained most of the beet-character compounds, while the acidic and phenolic fractions contained desirable constituents. Treatments can be tailored to exploit these attributes.

Carbon and cationic resins gave the best results for beet molasses, while XAD-2 and PVP were effective in reducing harsh and bitter flavor in cane molasses. Carbon is an excellent choice for wide spectrum component reduction in both beet and cane. Anionic resin was very poor for cane molasses.

The basic fraction also contained very pleasant nutty odors. These odors are strongly evident in some processing stages in

TABLE 5.--Adsorbent tests on cane molasses

Adsorbent	<u>Percent decrease in component</u>		<u>Flavor/odor characteristics</u>
	Phenol	Amino N	
None	---	---	Very bitter, harsh, cane molasses.
XAD-2	17.5	14.9	Slightly less bitter; mellowed; less acidic.
PVP	39.4	25.2	Most of bitter and harsh flavors removed; cane flavor remained; fruity odors are stronger.
Alumina	15.8	14.7	Little change.
Florisil	11.3	6.7	Little change.
Bentonite-NG	80.8	20.7	Very little flavor and odor; clay-like odor interferes.
Bentonite-160	17.8	9.4	Little change; clay-like odor interferes.
958 (NaCl)	14.3	0	Extremely harsh and bitter; acidic and fruity flavors are gone; very unpleasant.
118H (HCl)	0	54.2	More acidic; strong green-cane odor; not as strong.
118H (NaCl)	38.7	37.0	More acidic; much of bitter flavors removed.
Carbon	12.8	30.1	Improved flavor; less bitter; caramel odor and flavor; some green-cane; mild molasses flavor.

beet refining, and it may be possible that a syrup with nutty character could be marketed as a specialty product. In one beet factory, the odor of several process samples contained distinctive odors:

Diffusion juice	Raw beet, earthy
Thin juice from heater	Processed beet (different from raw beet)
Evaporator thick juice	Nutty
Std. liquor to conc.	Strong nutty odor and flavor
Std. liquor from conc.	Faint beet, nutty, and caramel
#1 green	Faint nutty
#2 green	Meaty, nutty
2nd green treated	Meaty, brothy

The special case of caramel-brown-burnt sugar compounds. It was noted that ion exchange resins are not generally efficient in removing compounds responsible for caramel odor, which can be a decided advantage in some applications and a disadvantage in others; for example, in manufacture of non-cola beverages, where a caramel flavor would be undesirable. Anionic exchangers would remove more of this class of compound than cationic resins because of these compounds' acidic character.

Batch resin treatment of three compounds used as models for caramel flavor gave the following results:

Percent removed by resin

Resin	Maltol	Maple Lactone	Vanillin
118H (NaCl)	21.2	3.2	9.2
118H (HCl)	23.7	5.6	8.4
958 (NaCl)	13.5	8.1	33.4

Conditions for the above tests consisted of 50 ml of solutions containing 50-70 ppm of compound in water, treated with 1 g wetted adsorbent for 30 min with shaking at 28° C.

ACKNOWLEDGMENTS

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DISCUSSION

L. Anhauser, Imperial Sugar - I have two questions. This is the first time I've seen "chocolate" flavor on a slide. Is that something you discovered recently?

Godshall - Chocolate flavor has been researched quite extensively. It is similar to coffee in that 300 or more compounds have been identified and no one single constituent is responsible for the flavor. Coffee and chocolate have undergone processing which is very similar to what occurs during sugar processing in that there is carbohydrate breakdown.

Anhauser - You found it only in beet?

Godshall - That is right. We have not found it in cane, but we do encounter it with beet sugar, and I think it has to do with the botanical origin. There are different compounds in beet, especially proteins and sulfur compounds that react with carbohydrates to produce something that smells just like chocolate. I have been told by beet processors that they can smell this odor in certain strata or levels of the plant. We noticed the odor during steam distillation of beet molasses. The molasses itself did not smell like chocolate, but the vent vapors exiting from the distillation had strong chocolate odor. This was confirmed by several observers.

Anhauser - Years ago, I tasted a brown sugar with chocolate flavor in it, and it is nice to know where it came from.

The other question I had: Would you care to comment on the effect of bone char on cane molasses since none of your slides referred to bone char, and several of us in the audience are interested in this from the food flavor usage standpoint?

Godshall - Bone char would probably have a broad-spectrum effect on the removal of flavor and odor compounds. In order to get a realistic picture of how bone char would work, we would have to use an elevated temperature. We do plan to look at it. In some preliminary work with bone char at room temperature, we found flavor improvement.

SURE: A NEW SUGAR DECOLORIZATION PROCESS

Dieter Frank, Lincoln D. Metcalfe, and John Park

AKZO CHEMIE AMERICA

Central Research Laboratory, McCook, Illinois

INTRODUCTION

At the SIT meeting last May in Baltimore we first reported about a new process for sugar decolorization. The basics of this new process - SURE as we call it - have been discussed in details at that time and I would like to include our SIT paper as reference here (Frank 1986).

The key to this new technology is a proprietary new microporous polymer ACCUREL (Castro 1985). It can be made of polypropylene or polyethylene. As can be seen from the electron micrograph (fig.1) it has a very regular microporous structure of typically 75% void volume. Its cells of 5 to 10 microns are connected by pores of 0.5 to 1 micron diameter. Its truly outstanding feature is the very high internal surface area of approximately 90 m²/gram that allows us to adsorb a variety of organic species from their solutions. Due to the existence of strong physical surface forces these adsorbed organics show very little leaching off the substrate. If the adsorbed chemicals are charged the "loaded" microporous resin can be used to remove species of the opposite charge from solutions.

As sorbents are widely used in the sugar industry a comparison of their physical properties may be appropriate (Meade 1985). One can easily see that ACCUREL has the highest porosity, the largest pores but a comparatively high surface area. This makes it very suited for applications where low resistance to flow (i.e. high through-put) is desired. (Table 1)

WATER SOLUBLE QUATERNARY AMMONIUM SALTS

In order to remove color bodies (mainly phenolics) from aqueous sugar solutions three conditions had to be met:

- * The active chemical had to be a cationic species.
- * It had to show affinity to the hydrophobic polymer matrix.
- * It had to be water soluble and wet the polymer.

From fig.2 you can see that the choice is rather limited. We are mainly dealing with mono-alkyl ethoxylated and monoalkyl trimethyl ammonium salts. The third type shown is a new group of proprietary quaternary ammonium compounds. All three types are

completely water miscible over the whole concentration range.

Table 1.--Structure of Sorbents

Type	Porosity %	Pores microns	Surface m ² /g
ACCUREL	75	0.5	90
Ion Exchange	20	.05	20
Bone Char		.001-10	50-80
Activ. Carbon	38	.01	1000

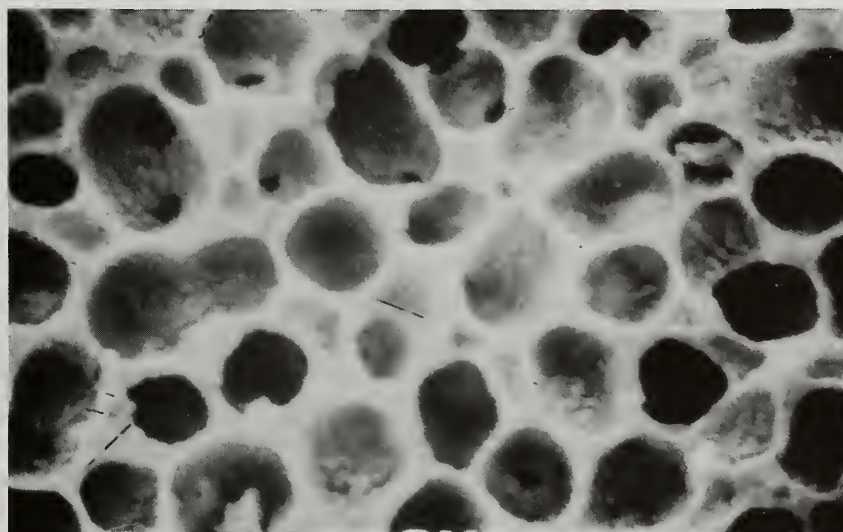


Figure 1.--Structure of ACCUREL

Due to their water miscibility all three types can be loaded onto ACCUREL from an aqueous solution. Their long fatty tails should provide the anchoring forces. Surprisingly only the type III quaternary ammonium salts showed good color removal from sugar solutions in our SURE systems. (Table 2)

Table 2.--Water Solubility and Color Removal

Type	R	Water solub. %	Color removal %
I	Coco	100	6
II	Tallow	>60	20
III	a	100	80
	b	100	75

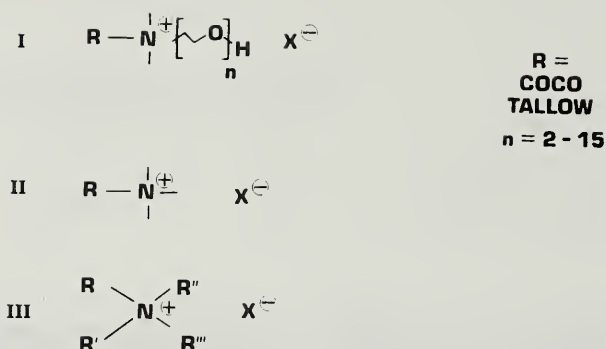


Figure 2.--Water soluble quaternaries

A film wetting test, developed in our laboratories, illustrates the differences clearly. Using ACCUREL film, we measured wetting rates for the various cationics by applying a known amount of quaternary to a known area and measuring the time it takes for the solution to be completely soaked into the film (to dryness).

Figure 3 shows plots of wetting rates versus surfactant units applied. The three types of quaternaries showed completely different wetting behaviour.

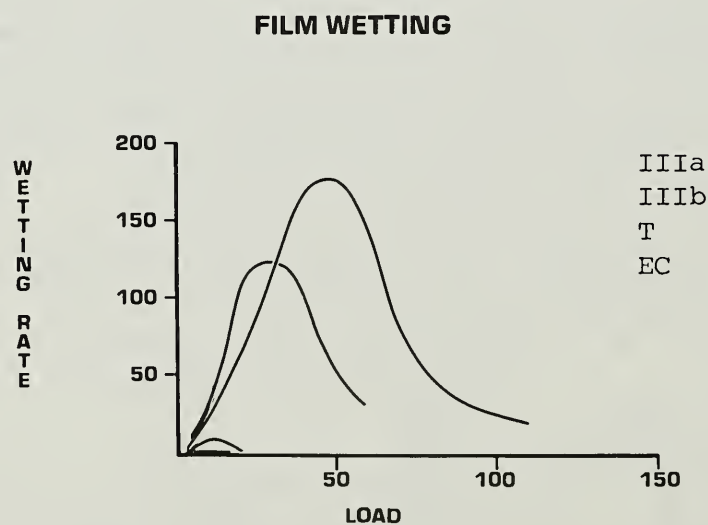


Figure 3.--Wetting rates

- * A sharp maximum exists for the two quaternaries that showed good color removal (III a,b).
- * Only a small maximum can be found for the marginal performer Arquad T.
- * A flat wetting rate appeared for the non-performers of the Ethoquad type.
- * Wetting rates of the type III quaternaries are about one hundred times greater than the rates of the standard commercial compounds.

This wetting test thus provides us with a predictive tool to select the best quaternary for the SURE process.

BATCH EXPERIMENTS

In order to get a better understanding of the mechanism of the SURE process and also to develop some process parameters a series of batch experiments was run first. A certain amount of ACCUREL powder was loaded with the quaternary IIIb and stirred with a volume of sugar solution equivalent to 15 bed volumes throughput if a column system would have been used. The amount of color (in ICU) removed was plotted versus time of mixing. Figure 4 shows that maximum color removal was achieved in less than 30 minutes. This would convert into space velocities of approximately 2 BV/hr.

In spite of the fact that the same amount of quaternary and ACCUREL was used for the three experiments, the absolute amount of color bodies removed decreases from 1800 to 350 and to 420 ICU for feed colors of 2700, 1040, and 480, respectively.

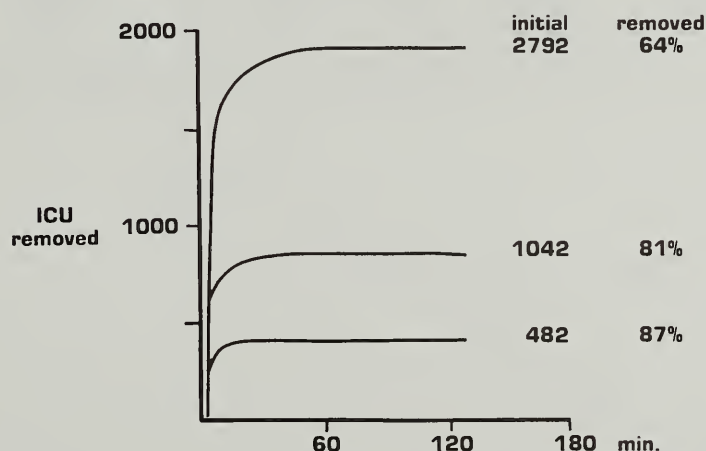


Figure 4.--Batch experiments

This phenomenon caused a little excursion into the field of adsorption phenomena. One of the basic theories was developed by Langmuir who first described an isotherm "that applies to adsorption on completely homogeneous surfaces, with negligible interaction between adsorbed molecules" (Perry 1973).

The Langmuir isotherm $q = \frac{Q K c}{1 + K c}$ (E.1)

relates q =concentration of adsorbed solute on the sorbent

c =concentration of solute in feed solution

Q =asymptotic maximum solid phase concentration
all in (mMol/g)

K =equilibrium constant (ml/mMol)

From a series of 16 batch experiments covering the color range between 566 and 4104 ICU and using 500 as an average molecular weight for the phenolic color compounds, the data of table 3 was generated.

Table 3.--Langmuir Isotherm

ICU Soln.	ICU Remov.	$1/c_e$	$1/q_e$	c_e mMol/g	q_e
128	438	30269	61.2	3.3×10^{-5}	0.016
155	480	24996	55.8	4.0	.018
164	482	23624	55.6	4.2	.018
349	671	11101	39.9	9.0	.025
509	953	7611	28.1	13.1	.035
744	1326	5207	20.2	19.2	.049
820	1450	4724	18.5	21.1	.054
1286	1540	3012	17.4	33.2	.057
1230	1616	3149	16.6	31.7	.060
1644	1564	2356	17.1	42.4	.058
1882	1768	2058	15.1	48.6	.066
2731	1245	1418	21.5	70.5	.046
2381	1723	1627	15.5	61.4	.064

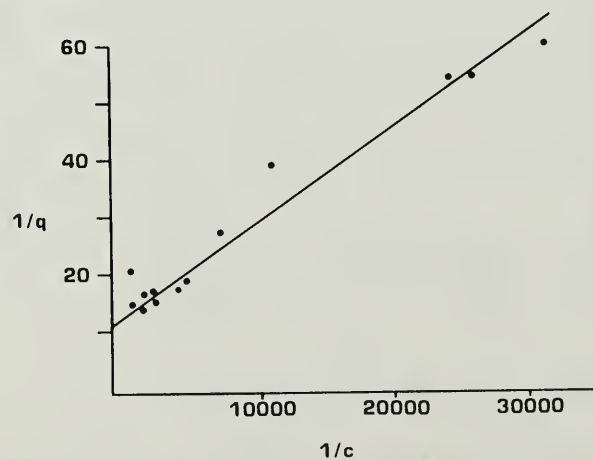


Figure 5.--Langmuir adsorption isotherm, linear plot

Plotting $1/q$ versus $1/c$ as done in fig.5 allows us to determine the two constants $Q=0.087$ and $K= 6385$.

The Langmuir isotherm thus becomes in numeric terms

$$q = \frac{555*c}{1+6385*c} \quad (E.2)$$

A plot of the isotherm shows very good agreement between calculated and measured equilibrium concentrations (fig.6).

These equilibrium concentrations have little value unless one converts them into feed and product colors using the mass balance

$$c_i*S=c_e*S+q_e*A \quad (E.3)$$

where c_i stands for feed color, c_e is product color, and q_e represents color adsorbed (i.e. removed). S and A are the amount of solution and sorbent used.

In order to convert concentrations in mMol/g into color units we used an average conversion factor of 7.75 ICU/ppm of phenolics. This number was derived from data reported by Clarke (1985) and has served us well so far.

After all these mathematical manipulations the Langmuir isotherm can be drawn in units of practical value: feed ICU and product ICU.

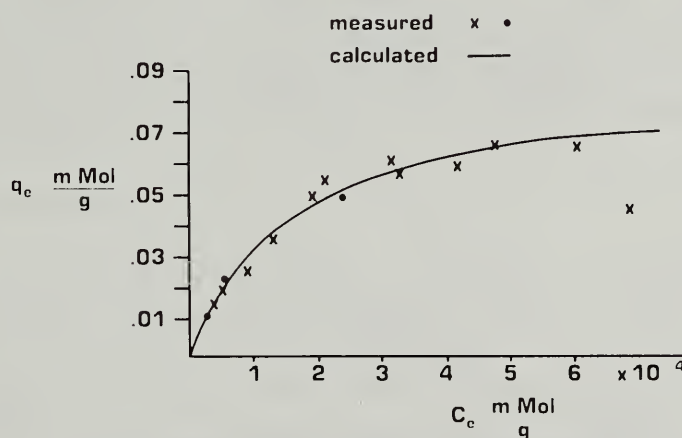


Figure 6.--Langmuir adsorption isotherm

The isotherm as it is shown in figure 7 still correlates **equilibrium concentrations** but is of great importance as it allows us to calculate the minimum number of stages necessary if a certain color removal has been set as target.

Figure 8 is certainly familiar to many of you. It resembles a McCabe Thiele diagram as it is used for distillation processes.

In the example shown a feed color of 4000 ICU will, after

passing through a three stage system, produce a product color of 250 ICU.

It should be re-emphasized again, this is true only for batch equilibrium systems and does not yet teach how much sugar solution (S in grams) can be treated by a given amount of adsorbent (A in grams).

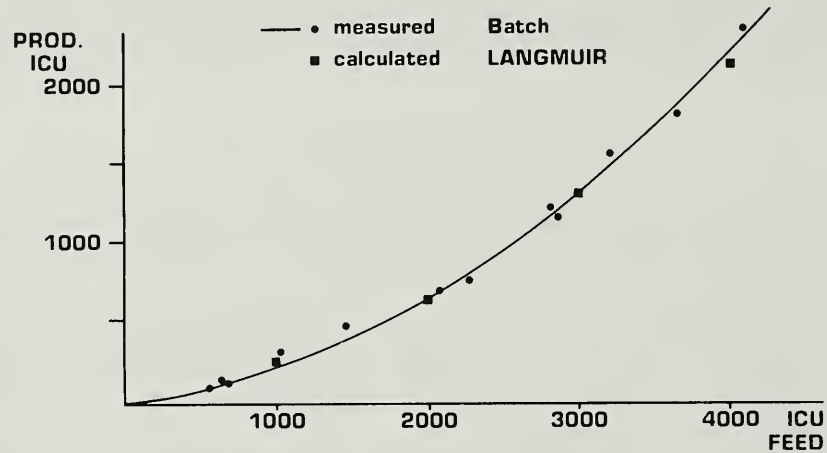


Figure 7.--Correlation of product and feed color

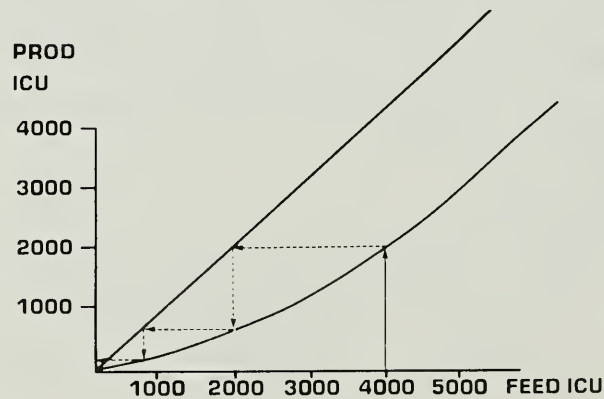


Figure 8.--Langmuir isotherm, number of stages

Equation 2 can be modified using the mass balance equation (3) and the conversion factor mentioned above to finally arrive at the most general equation, correlating feed and product color with amount of solution treated and sorbent used.

$$A = S * \frac{(1 + 1.647 * 10^{-3} * C_E) * (C_I - C_E)}{555 * C_E} \quad (E.4)$$

Using this equation the experiments of table 3 were used to compare actual used amounts of sorbent and solution with the calculated ones. Table 3a shows that, except for two cases, excellent agreement could be observed.

Table 3a.--Amount of sorbent

Feed ICU	Prod. ICU	Solut. g	g Sorbent calc. used		Dev. %
566	128	650	4.85	4.5	+7.8
635	155		4.55		+1.1
646	164		4.37		-2.9
1020	349		3.54		-11.3
1462	509		4.03		-10.4
2070	744		4.64		+3.1
2270	820		4.86		+8.0
2826	1286		4.37		-2.9
2846	1230		4.65		+3.3
3208	1644		4.13		-9.2
3650	1882		4.51		+ .2
3976	2731		2.93		-34.9
4104	2381		4.17		-8.4
482	67	2750	34.07	27.0	+26.2
1042	192		28.87		+6.9
2792	902		25.8		-4.4

We now believe that the mechanism of the SURE process is basically an adsorption of phenolics on a quaternary (mono?) layer supported by the microporous polymer. Our future work will address the special situation using multi stage column systems in more depth.

COLUMN EXPERIMENTS

For all practical purposes the column experiments are much more important for evaluating the merits of the SURE process. In consecutive order, the following steps are involved and have been optimized in our laboratory (table 4):

LOADING: We typically use 1.5 bed volumes of an aqueous solution of the quaternary. This solution is being circulated at 40 BV/hr through the column containing the dry ACCUREL particles.

RINSING: One bed volume of water is being used to rinse out excess of quaternary (25 BV/hr). The rinse solution can be re-used as loading solution after adding the appropriate make up amount of quaternary. Although not yet proven it may be of advantage to use sugar solution for rinsing out the charging solution. This would avoid any dilution effects in the early phases of the operation. This sugar rinse eluate can easily be used for the next loading step.

OPERATION: Sugar solution of 65 Brix at 60 to 75 degrees

Celsius is pumped through the column in upflow mode. Depending on the feed color level, exhaustion of the column is being observed between 14 bed volumes (for 5000 ICU) and 100 bed volumes (for 800 ICU). Typical space velocities are from 3 to 40 BV/hr. These very high velocities are one of the outstanding features of the SURE system. It goes without saying that velocities of 40 BV/hr are only attainable with low color feeds.

SWEETENING OFF: After exhaustion, remaining sugar values are being rinsed out using one bed volume of water at a rate of 12 BV/hr. This eluate can either be used for affination or goes directly to evaporation.

REGENERATION: The SURE columns can easily be regenerated using caustic brine solutions as used in the regeneration of ion exchange columns. Typically 1.5 bed volumes of brine are being used at a rate of 3 BV/hr.

RINSING: Rinsing out remaining brine regenerant completes the cycle. Two bed volumes of water at 3-12 BV/hr are sufficient to prepare the column for reloading. The relatively low flow resistance of the ACCUREL bed and the high porosity of the polymer (as a reminder: 75%, 0.5 micron pores) allow us to run most of these steps at comparatively high space velocities.

Table 4.--Process steps

Operation	Amount BV	Type	Rate BV/Hr
Loading	1.5	IIIb/Water	40
Rinsing	1.0	Water	25
Decoloriz.	3-45	65% Sugar	3-40
Sweet. off	1.0	Water	12
Regenerat.	1.5	NaCl/NaOH	3
Rinsing	2.0	Water	3-12

A typical single column run is shown in fig.9. Here we used a 65 Brix solution of 3150 ICU that was passed through one single column of 200 ml bed volume. 31 grams of ACCUREL sorbent was used. The dots in fig.9 are the measured color removals up to 15000 grams treated. This is equivalent to 50 bed volumes.

This run was used to compare column performance with calculated color removals using the Langmuir isotherm that was derived from our batch experiments shown as a solid line in fig.9. As can be seen, reasonable agreement is found. The column performance is slightly below batch color removal at the early stages but surpasses the batch once large volumes have been treated. A sugar solution of 4520 ICU feed color showed a very similar pattern (fig.10).

There is one important difference between columns and batches that could possibly explain some of the differences observed. Columns operate under forced flow conditions which allow us to access even the inner parts of the sorbent particles at high space velocities.

A typical space velocity of 25 BV/hr amounts to 2.4 minutes residence time. Batches on the other hand have 30 minutes of residence time but flow into the particles is diffusion limited and thus slow.

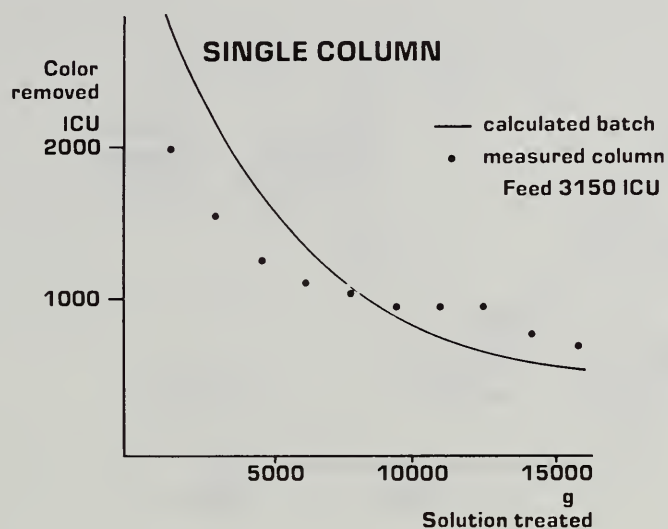


Figure 9.--Comparison of color removal

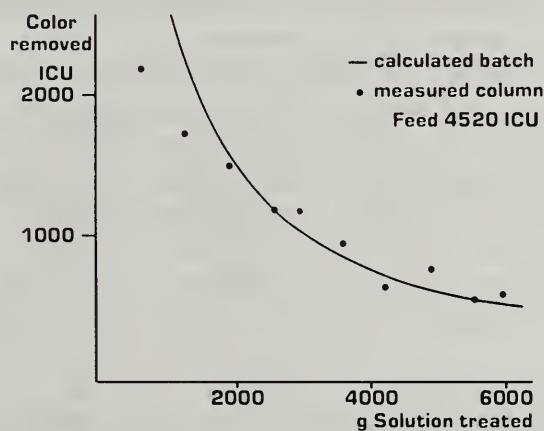


Figure 10.--Comparison of color removal

THREE STAGE COLUMN EXPERIMENTS

In order to extend the cycle length, more than one single column has to be used in series. In spite of their differences, the batch experiments have shown indications that three columns in series may yield an optimal performance.

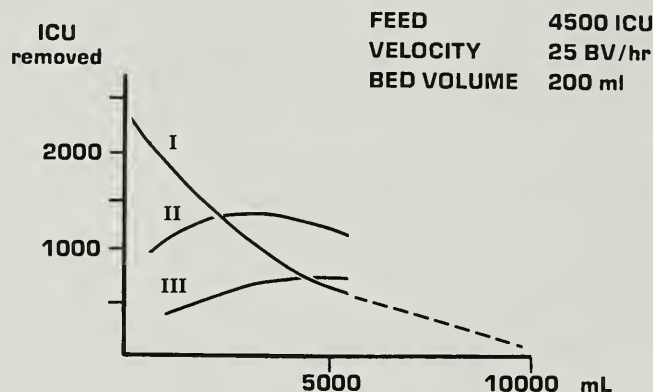


Figure 11.--Three stage column run

Fig.11 shows in graphic form an experiment which could be called typical although the incoming feed color was higher than most refiners may like to use.

Starting with a sugar solution of 4500 ICU the effluent from column I initially shows color removal of 2000 ICU. It drops steadily to 600 ICU when 26 bed volumes have passed the column. Space velocity in this experiment was as high as 25 BV/hr.

Column II shows an initial color removal of 1000 ICU. This increases to a maximum of 1400 ICU about half way through the run and stays high to the end. Not surprisingly, column III is almost dormant during the first half of the run but starts to contribute to the over all color removal when the effluent from column II starts to challenge column III with higher and higher color levels.

Due to the exhaustion of column I, the run had to be terminated at 26 bed volumes. It is obvious from the dotted line extrapolation that columns II and III could have been used for at least another 25 bed volumes. This naturally leads to a multi column system with column rotation. A fresh column IV would go into service when column I goes into regeneration.

Column Rotation

In an experiment with five columns of 200 ml bed volume each, a sugar solution of 4580 ICU feed color was run through columns #1,2, and 3 to a total throughput of 14 bed volumes (=2800ml) at

a space velocity of 25 BV/hour. At this time column #1 was taken out of service and a fresh column #4 was added as the trailing column. And again after 14 bed volumes #2 (the now leading column) was taken out and #5 went in. Fig.12 shows that indeed the system continued to perform as expected.

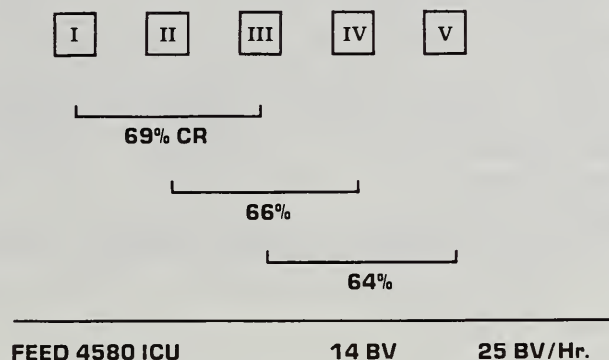


Figure 12.--Column rotation

The slight drop from 69 to 66 and 64% color removal is due to the fact that steady state conditions are not reached before the third rotation. Normalizing these results by converting them into color removed times bed volumes treated (table 5) shows efficiency factors of approx. 42000 ICU*BV.

Table 5.--Column rotation

Columns used	Color removed	Bed volumes	Effic. ICU*BV
I-III	3160	14	44240
II-IV	3023	14	42322
III-V	2931	14	41034

Effect of a Trailing Carbon Column

The SURE system, in spite of its merits and interesting performance, is not meant to be a free standing unit. It can not be run as the sole color removing system. One reason is that the product sugar solution will or may contain some quaternary ammonium salt that was leached off the columns. Another reason is that according to our present understanding of the process, mainly phenolic color bodies are being removed by the quaternary. The remaining color after passing the SURE column can further be removed by a carbon bed which also will lower any residual quaternary to acceptable levels. This carbon column can be much smaller than the conventional carbon beds used when the

bulk of the color removal has to be achieved using carbon columns. We were also pleasantly surprised when we found that the trailing carbon bed could be run at higher space velocities (up to 6 BV/hr) and that the total throughput through a carbon bed could be as high as 22 bed volumes. Both of these results compare favorably with a conventional bone char bed (< 1 BV/hr and 15 BV). These results and the amount of Arquad IIIb in the processed sugar are shown in table 6.

Table 6.--Effect of a trailing carbon bed

	Before	After
Color (ICU)	700	45
Arquad (ppm)	30	.3
Talofloc (*)	n.a.	.64
(*) Tate & Lyle (1972)		

Sugars of Various Origins

Most of the data presented so far was generated using more or less standard raw sugars of a relatively low color. In order to test the limits of the SURE system we have run sugars of various origins through a three column system up to the almost incredible feed color level of 26,000 ICU. The results are shown in table 7.

Table 7.--Decolorization of various sugars

Origin	Feed ICU	Treated BV	Removed ICU	CR %
Brazil	1688	10	1495	88.6
S.Africa	4321	5	3937	91.1
Zimbabwe	6910	8	5826	84.3
Jamaica	7378	5	5863	79.5
Dom.Republic	11947	3	10530	88.1
Philippines	25808	5	22889	88.7

The results shown—although spectacular by % color removal—are more significant when shown in graphic form as done in fig.13. What we clearly see there is that over a certain range of bed volumes treated the color removal is linear and thus can be extrapolated to a certain end point. This end point can be calculated from the Langmuir equation in its form E.3 and/or from the amount of quaternary used in these columns. Without going through all the details table 8 summarizes these extrapolations.

Table 8.--Extrapolation of performance, various sugars

Orig.	Feed	BV	BV	Col.rem.	ICU*BV	
	ICU	act.	max.	extrap.	Actual	Extrap.
Brazil	1688	10	21.8	1300	14950	28340
S.Africa	4321	5	13.3	3700	19685	49210
Zimbabwe	6910	8	14.9	5000	46608	74500
Jamaica	7378	5	16.5	5000	29315	82500
Dom.Repub.	11947	3	9	9300	31590	83700
Philipp.	25808	5	5	22889	114445	114445

These efficiency factors in BV*ICU are an important number to measure the performance of a SURE system. We had earlier reported that a range of 40000 to 80000 had been observed depending on conditions. This series of experiments with very unusual sugars reconfirms these factors. Detailed graphic evaluation of these factors seem to show an asymptotic maximum performance factor that is around 110,000 ICU*BV.

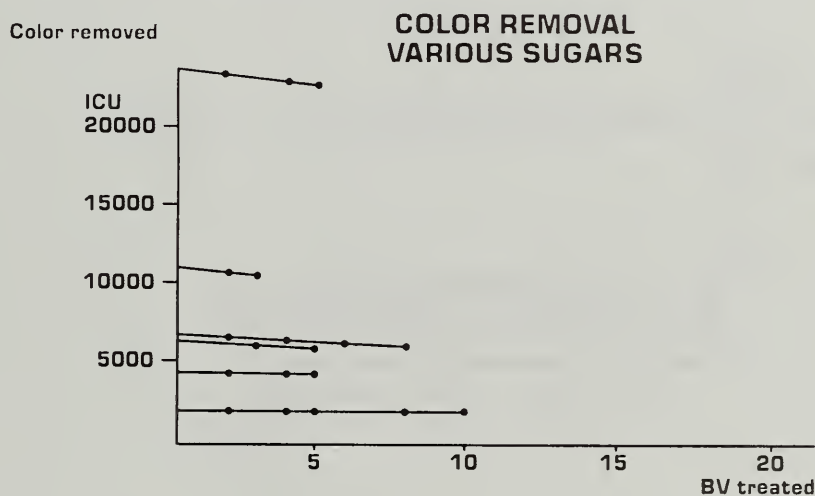


Figure 13.--Color removal and feed color

Technical Limits

Every process has its technical limitations. So does the SURE process. Let's look at these boundaries.

Temperature should not exceed 80 degrees Celsius. The delicate internal surface area of ACCUREL will undergo changes during long term exposure to high temperatures. No change of the surface area could be observed over 35 days at 60 degrees Celsius.

Lifetime of ACCUREL: Notwithstanding its temperature limitation, ACCUREL powder did not show any significant change in performance when run under standard conditions up to 35 cycles (table 9). Meanwhile we have continued these runs without any indication of structural changes.

Table 9.--Lifetime of ACCUREL

Cycle #	Feed color ICU	Color removal %
1	2230	83
23	2233	82
35	2070	75
2 Columns	30 Brix	15 BV 15 BV/Hr

Space velocity: Whereas regeneration should be done at relatively low velocities of typically 3 BV/hr, the actual operation allows space velocities up to 40 BV/hr. Fig.14 shows that the process is relatively unaffected by space velocities. The drop in relative color removal from 76 to 71% between 7 and 40 BV/hr may well be within experimental error.

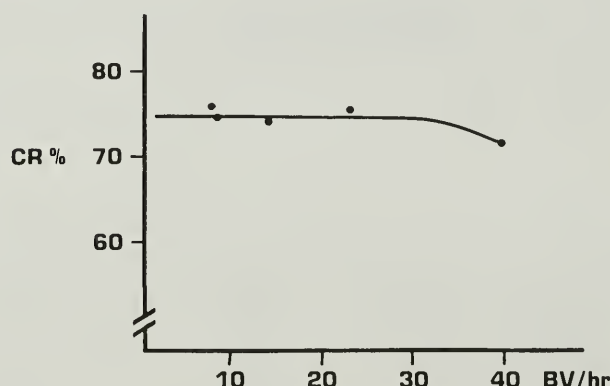


Figure 14.--Color removal vs. space velocity

Pressure drop in columns: We had reported earlier some very preliminary results on pressure drops in SURE columns. Pressure drop is, of course, of high importance for the engineering layout of our systems.

For beds filled with porous particles one would normally first use Darcy's equation for flow through porous beds. Since some of the constants used by Darcy are not known for our system, we used the Leva packed bed equation.

$$p = \frac{2 * f * G^2 * L * (1 - \epsilon)^{3-n}}{D * g * \rho * \phi^{3-n} * \epsilon^3} \quad (E.5)$$

In this equation most of the terms used can be taken from the literature or can be easily determined by iteration.

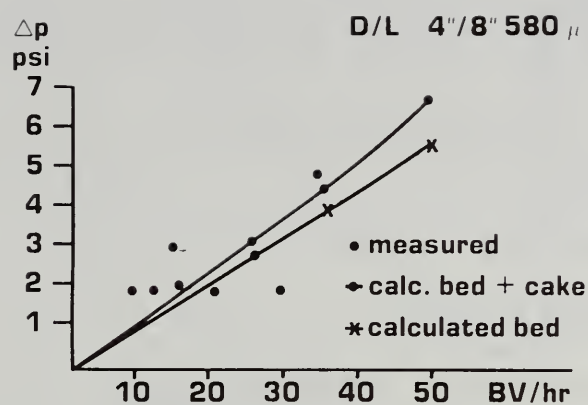


Figure 15.--Pressure drop vs. space velocity

**CAKE CONTRIBUTION TO
PRESSURE DROP IN A 6 FT. COLUMN**
at 15 BV/hr

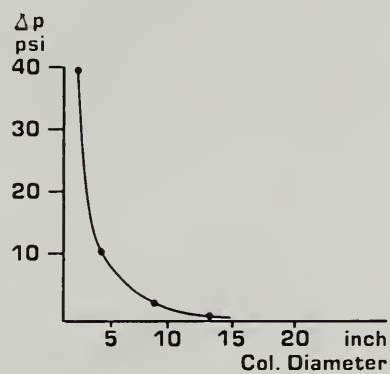


Figure 16.--Cake contribution to pressure drop

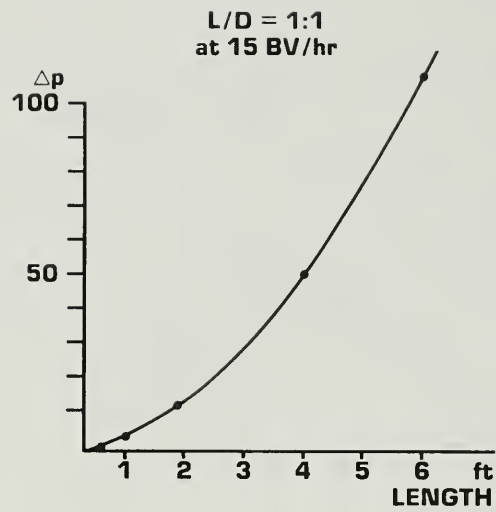


Figure 17.--Pressure drop vs. column length

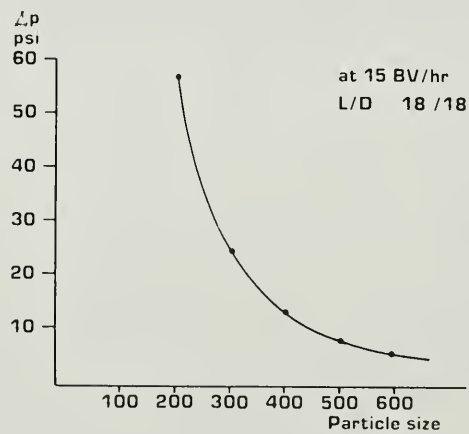


Figure 17a.--Pressure drop vs. particle size

The parameters used are

p=pressure drop along L

f=friction factor

G=superficial mass vel.

ϵ =void fraction

μ =fluid viscosity

D=particle diameter

g=32.17 (constant)

ϕ =shape factor

ρ =fluid density

n=exponent (from Reynolds No.)

A void fraction of 0.4 (which was found by iteration to be the best fit) accounts for some fractions of the total flow **through** rather than around the particles. Without going into all the details of the computations some results should be summarized here.

- * Pressure drop is directly proportional to space velocity (fig.15).
- * A small contribution from possible cake build up exists but becomes insignificant with larger bed diameters (fig.16).
- * Pressure drop increases by the second power of the column length (fig.17).
- * Particle size in the bed has a significant influence on pressure drop (fig.17a).

A good example for the validity of the Leva equation is shown in fig.18. The measured pressure drops in an 18/18 inch (L/D) prototype column fall practically on the line that was calculated using Leva's equation. This pressure drop will be lower when one switches to the larger resin particles that are now available to us. Color removal is not affected by particle size - at least not in the regions we are interested in (≤ 20 BV/hr and ≤ 600 micron diameter).

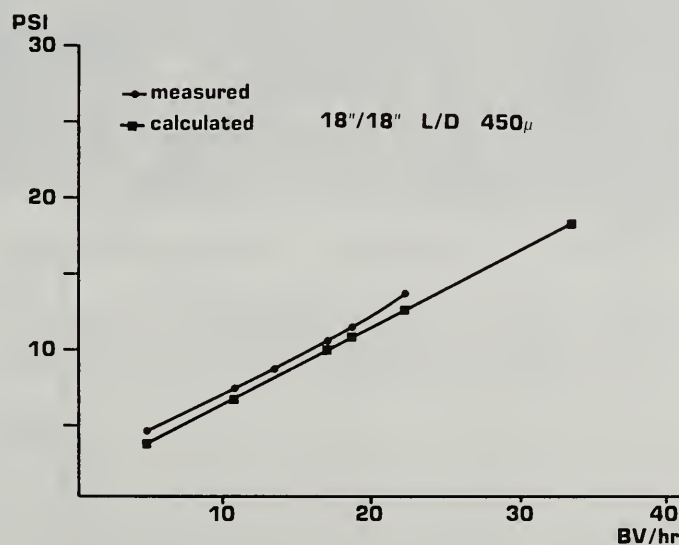


Figure 18.--Pressure drop vs. space velocity

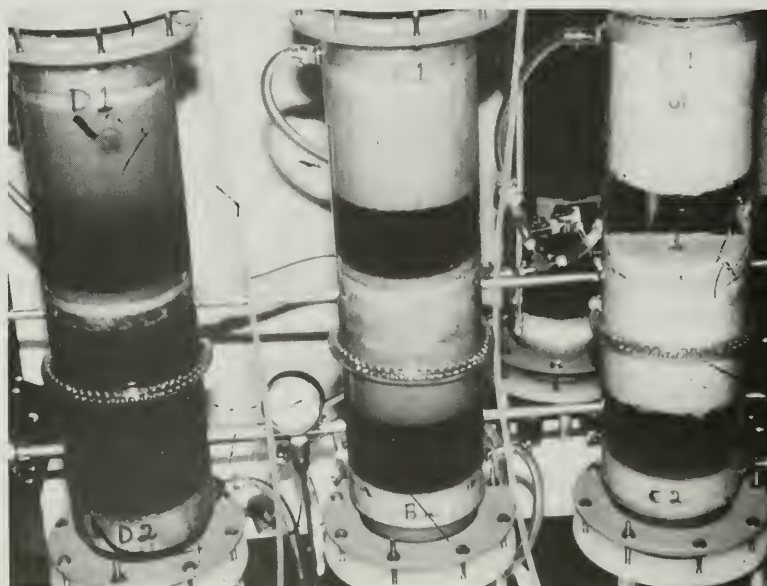
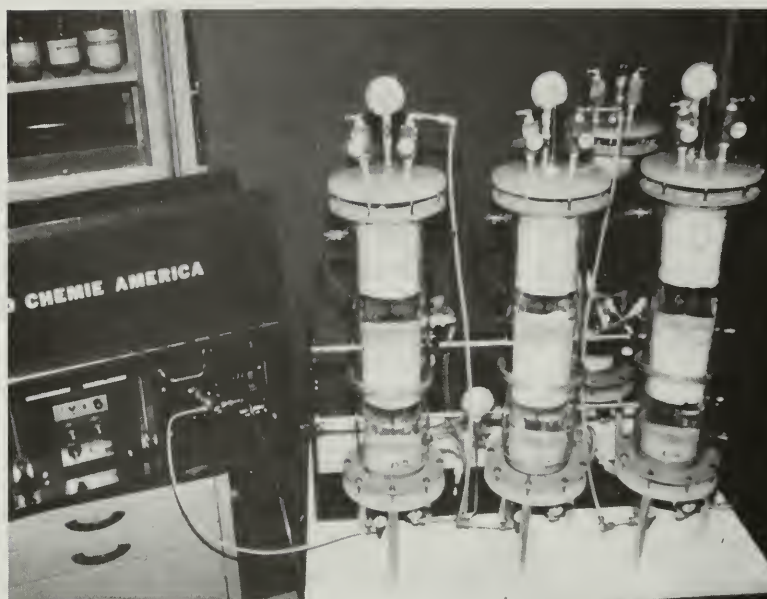


Figure 19.--Portable 3 column SURE unit

Upper photo: control unit (left) and columns (right)
 Lower photo: columns during processing

Geometry: Closely related to the expected pressure drop along the column is the diameter to height ratio of the column. We have worked with ratios of L/D from 1 to 4. The preferred ratio is 1:1. This avoids excessive pressure build-up.

Flow mode: An interesting phenomenon was observed. The "natural" downflow causes bed compaction much quicker than upflow. Upflow has another advantage insofar as during draining the bed will be loosened up.

RUNS WITH A PORTABLE PILOT UNIT

After most of the process optimization work had been done using our relatively small laboratory unit of three columns of 200 ml bed volume each, we started a modest scale-up to a unit as shown in fig.19. We use three columns of 1700 ml each. You can also see the trailing carbon column. The unit was intended to be used for laboratory runs as well as for trials under actual field conditions in sugar refineries. Last March we tested it in a refinery using various slip streams from their production. These runs can be summarized best in form of the following table 10:

Table 10.—Field runs with three columns

Feed:	Phosph.	Affin.	Remelt
Color, feed	1500	1700	3000
Color, prod.	380	289(*)	917
Color rem. %	74	83	69
BV treated	28	21	14
ICU*BV	31360	29631	29162

(*) 75 ICU after Carbon

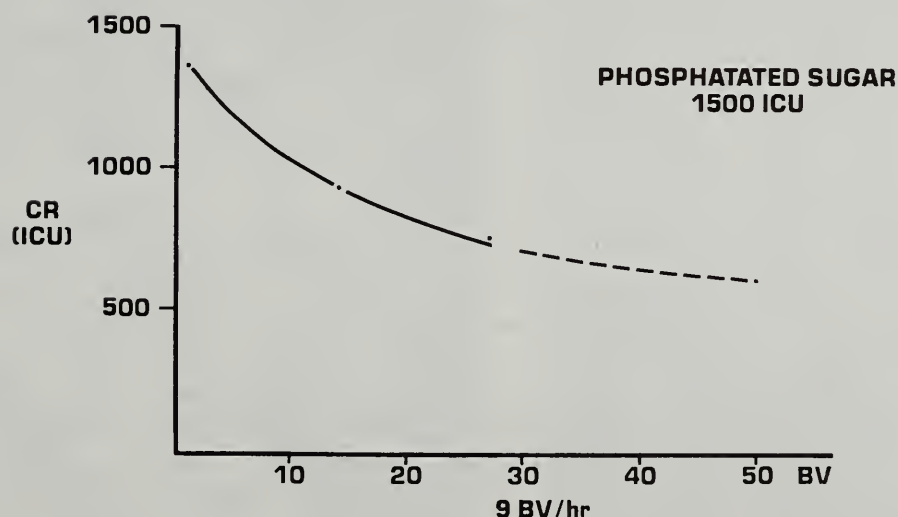


Figure 20.--Three column run

Experiment #1 was using phosphatated and filtered sugar of 1500 ICU. After 28 bed volumes throughput, the cumulative color in the product was 380 ICU (i.e. 74% color removal; fig. 20).

In the next run, we used affinated and filtered sugar. Its feed color level was 1700 ICU. Color in the collected product after 21 bed volumes was 289 ICU. Space velocity in these runs was 9 BV/hour. The trailing carbon bed (activated charcoal) was used to further treat this effluent. Due to the differences in attainable space velocities, this carbon treatment was done after the termination of the run. The color could be further reduced to a comfortable 75 ICU (cumulative after 10 bed volumes).

Experiment #3 was quite a challenge to the SURE system. Here we used remelt sugar of 3000 ICU. Remelt sugar contains not only plant produced but also process generated color bodies which are different in their chemical nature. In spite of all this, over a run of 14 bed volumes, we could lower the color to 917 ICU.

The last line in table 10 shows these results in a normalized way using $\text{ICU} \times \text{bed volumes}$. The numbers reported here vary only slightly with an average of 30050 $\text{ICU} \times \text{BV}$. This seems to indicate again that the mechanism of color removal in the SURE process is mainly a stoichiometric one.

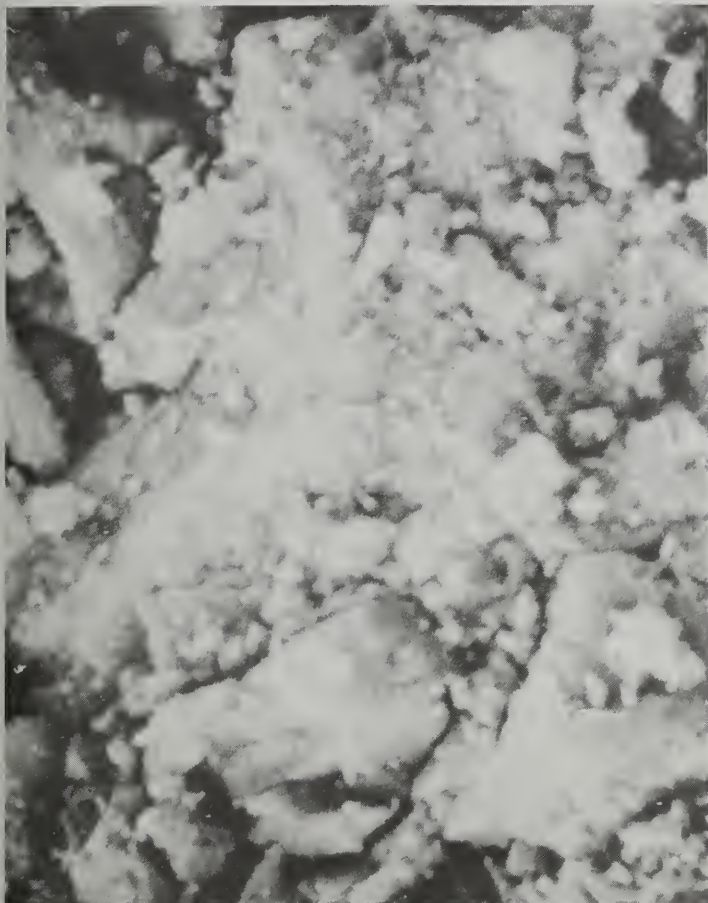
ADVANTAGES OF THE SURE PROCESS

Let me try to summarize various features of the SURE process and compare it with some of the standard operations in sugar refining.

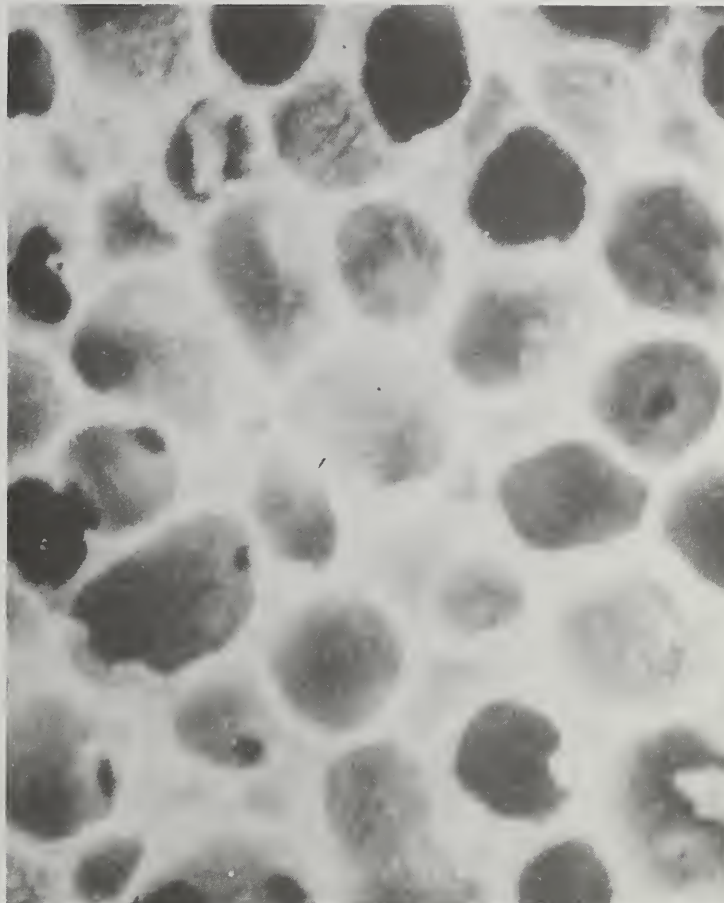
Space velocity: As can be seen from table 11, space velocities in operation but also in regeneration are greater in the SURE process than in ion exchange, carbon, and bone char operations. The very different type of porosity of ACCUREL, ion exchange resins, and carbon is the main factor in achievable space velocities. The photos in Fig. 21 show these differences clearly. At the same magnification, no pores can be seen for ion exchange resin and carbon. Access to the interior of the particles is mainly diffusion controlled.

Color Load: Table 11 also indicates that the color load can be much higher in the SURE process. Even incoming colors of 5000 ICU should not create any problem if one is willing to run shorter cycles as the column capacity is a pre-set constant.

Regeneration: As SURE in a certain sense is a distant relative of conventional ion exchange, regeneration uses the same chemicals and basic principles as known from ion exchange. Due to the physical differences between both systems regeneration can be done faster in the SURE process (e.g. 3 versus 1 bed volume /hour).



Activated Carbon, 1050X



ACCUREL, 1050X



Bone Char, 1050X



Ion Exchange Resin, 1050X

Figure 21.--Structure of sorbents

Table 11.--Comparison of processes

Proc. step	SURE	Ion exch.	Act. carbon	Bone char
Decolorization				
BV/Cycle	28	50	85	15
BV/Hr	9	2	.12	.25
Hrs/Cycle	3	25	720	60
Regeneration				
PV/Hr	3	1		
Hrs	.5	.5	20	12
Rinse Hrs	.5	.5	-	-
Total Hrs/Cycle	5	31	750	72
Feed Color ICU	1500	900	1000	1000
Color Rem. %	75	85	80	90
" " ICU	1120	765	800	900
Efficiency				
BV*ICU	31000	38000	68000	14000
Yield				
BV*ICU/Hr	6200	1245	90	194

Unit Size: Due to the aforementioned differences in space velocities, the size of a SURE unit can be significantly smaller than ion exchange or carbon beds. Table 11 tries to compare what could be called typical conditions and performance of a SURE system versus ion exchange, bone char, or activated carbon treatment. Total throughput per cycle is not too different for the four systems with SURE being on the low end (28 bed volumes) and activated carbon (85 BV) having the highest capacity. Total time for one cycle, however, is only 5 hours for SURE, 31 hours for ion exchange, 72 hours for bone char, and 750 hours for activated carbon. These times include everything from loading through sweetening off and regeneration. Taking these numbers and normalizing them as done before, we arrive at 31000 ICU*BV for the SURE process versus 38000 for ion exchange. Bone char and carbon are on the low and high end of the range. One can also calculate a "yield" factor, measured as ICU removed times bed volumes treated per hour (ICU*BV/Hr). Here we see that the four systems are different by almost an order of magnitude. SURE calculates to 6200, ion exchange 1245, bone char 194, and activated carbon as low as 90 ICU*BV/Hr. These numbers convert directly into very low space requirements for a SURE system.

Environmental Issues: The use of Arquad IIIb certainly has to be FDA approved. Discharge of spent regenerant could cause some problems in certain locations not using ion exchange as of now. We are currently working on some alternatives to caustic brine. Other avenues to be pursued are certain recycle loops which could eliminate or at least lower the environmental discharge load.

SURE IN THE SUGAR REFINERY

In spite of the fact that we have spent considerable time and effort during the last few years on learning as much as possible about the modern sugar industry in no way do we claim to be experts in this field. From all we know as of today, we believe that SURE can replace conventional ion exchange and parts of the bone char or carbon treatment. From recent discussions we had in the field, it seems possible to use a SURE unit as an "Insert" to increase capacity of refineries which mainly depend on bone char and activated carbon decolorization. In this scenario a SURE unit would follow affination and discharge a much lower color syrup into bone char or carbon treatment.

One other aspect has to be considered. Our most recent positive experiences with extremely dark raw sugars and also the good results with remelt sugar merit a further investigation. It has been reported (James 1986) that recycle of color bodies with the remelt stream can cause unnecessary problems to the refiners.

We will also continue to test our process in a few refineries using our small portable unit while a larger (but also mobile) pilot plant is being built in the first half of next year.

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DISCUSSION

N. W. Broughton, British Sugar - Thank you, Dr. Frank, for a most interesting paper. I have two questions. First of all, have you any knowledge of the application of this process in the beet sugar industry as opposed to the cane industry?

Secondly, you have shown in your last parameter, yield, that there is a several-fold improvement in yield relative to conventional processes. Can you tell us what you estimate the cost to be relative to those conventional processes?

Frank - The first question is very easy. The second is not my department, but I will answer both.

Yes, we have experience with beet sugar. It is not as extensive as with cane, as it is very hard to get a good representative beet sample. With the two we have treated, we got about half the performance we have reported for cane. That means when I report 40,000 ICU bed volumes for cane, I got about 20,000 to 25,000 for beet, which is not bad, knowing that the color bodies in beet are different from the color bodies in cane. We are currently trying to get more samples to devote more efforts to beet treatment, as we have covered the cane area pretty well.

The second question about costs: We are convinced that we are competitive with the existing systems, but I cannot give you numbers here.

J. Polack, Audubon Sugar Institute - I was wondering about the Accurel resin. Is it an article of commerce that we can buy or is it only available through the use of the SURE process?

Frank - Yes. It is an article of commerce. It is traded mainly as membranes, but we have started to make powders and small particles in all sizes. It is handled by one of our sister divisions.

EVALUATION OF CARBON-CHAR ADMIXTURES

Brian Dewar and Andrew Ho

Redpath Sugars

In the late 1970s Redpath experienced a need to improve the efficiency of its char house. While our char condition was very good, increasing melt rates and a need to char certain syrups for soft sugars were adding to the color loading. As a result, the company began a study of various decolorizing processes to find more efficient alternatives. Unfortunately, the methods then available usually involved large capital expenditures and major system changes.

In early 1979, however, Calgon introduced a method of mixing animal bone char and granular carbon as a replacement for the pure bone char we had been using. The admixture was composed of between 80% and 85% char and 15% and 20% granular carbon by weight.

Exploratory studies by Calgon found that the admixture's decolorization capacity was not only substantially better than bone char alone, but service life could be extended an estimated 1.8 times (1). It appeared to Redpath that such an admixture could give the char house the extra capacity it needed at a minimal capital cost.

We conducted preliminary tests late in 1981. Based on satisfactory results, we introduced granular carbon to all 12 of our cisterns in June of 1982 in an adsorbent mixture of 85% char to 15% granular carbon by weight. By adopting the admixture, we hoped to give an extra boost to the system. Because our char conditions had been consistently good historically, we took care to monitor the admixture constantly. It was important to maintain the integrity of the bone char portion of the mixture since it represented the bulk of our adsorbent.

As with any new process, problems arose shortly after we adopted the admixture technique. We began to experience difficulties with performance, regeneration, ash removal and dust. Initially, there was an immediate gain in the overall percent decolor-

ization efficiency, as we had expected. However, this appeared to be followed by a gradual decrease in the efficiency over time, which we had not expected (Graph 1).

As a result, it became critical for us to thoroughly evaluate the admixture. To do this, a method had to be designed to separate the two components so they could be tested. It was only after adequate separation that the quantity and the quality of each component could be determined.

However, while new bone char and new granular carbon have different physical and chemical properties, during the dynamic process of service and regeneration these differences become masked. Particle sizes change due to different attrition rates, bulk densities change due to service and available surface areas change due to different adsorption rates and unbalanced regeneration. As a result, the rate of change for each adsorbent is not the same. To make matters worse, masking is a degenerative process. As the service admixture grows older, masking increases and separation becomes even more difficult.

Various techniques had been developed including the use of different density media, electrostatic charges (2), air fluidization, water fluidization and separation by screening. Unfortunately, for each method there were limiting factors. Most methods achieved relative success only when using new bone char and new granular carbon fractions. With new admixture, physical and chemical properties are as yet unmasked by usage, and still sufficiently different to allow separation. But as the admixture's length of service increased, the ability of these methods to perform an adequate separation declined.

In July of 1982, Redpath started to investigate different methods of separation. We were looking for a method that was practical enough to use our existing laboratory capabilities while providing meaningful results. While a high degree of accuracy was desirable, we did not need an absolute scientific solution.

We first tried the "Settling Method," which draws upon the difference in bulk densities of the two adsorbents. A 2 1/4" diameter and a 24" long column was filled with a liquid medium, and a sample of the "synthetic" admixture (20% by weight of granular carbon and 80% by weight of bone char) was added to the top of the column. The sample was allowed to settle and the separation observed.

As a medium, we first tried ethanol, then liquids of gradually increasing density. Separation results are shown in Table 1. Initially, very little separation was observed although a trend was evident. It appeared that the higher the density of the medium, the better the separation.

In 1984, we asked Tate & Lyle's Research Group to help find a satisfactory separation technique. They developed a settling method using a saturated zinc iodide solution (Table 2). While this technique indicated a considerable improvement in separation, there was a drawback. Zinc iodide solution at a density greater than two is very dark, almost black. This made visual observation of the separation extremely difficult.

Tate & Lyle also achieved a good separation from a mixture of sodium iodide and zinc iodide solution with a density of 2.15 gm/ml (Table 3). Unfortunately this method also had drawbacks. Bone char is a very good adsorbent of zinc, and iodine tends to bind to the two adsorbents. Accurate analysis of the separated adsorbents was, as a result, impossible.

At Redpath, we continued to experiment as well, trying a method using air fluidization. This technique has several advantages. First, after separation no drying or conditioning is necessary before analysis. Second, flow rates can be easily controlled. Third, there are few impurities in the air to affect the adsorptive capacity of the separated fractions. Results of our air fluidization experiment are shown in Table 4.

While this technique appears to work, there is one big disadvantage. During the expansion of the bed, the particles of the samples collide with one another and the wall of the column, causing some attrition and the formation of dust. Damage to the sample created doubts about the validity of the separated samples.

Calgon was also working on the separation problem. As a result of their experiments, they recommended a water fluidization bed technique which apparently met the various criteria necessary for our use. It appeared to provide relatively good separation, produce workable sample sizes, did not pollute the separated adsorbents, is fairly reproducible and used standard laboratory equipment. Details of the procedure are shown in the Appendix.

We tried the Calgon separation method on a variety of adsorbents, including pure granular carbon, pure stock bone char, heavy bone char, admixtures of bone char and granular carbon with known ratios and the granular carbon and bone char admixture from our char house. After separation, each column was sectioned into several fractions. These fractions were then examined using the following methods (Table 5 - Table 9):

1. The samples were checked visually by macroscope for the presence of bone char or granular carbon. Under the macroscope, bone char particles appeared irregular and grayish with a shiny luster. New granular carbon particles were dull black and cubic in shape. Granular carbon particles in use became more rounded over time.

2. The bulk density of each fraction was ascertained (Graphs 2 - 5).
3. The particle size of each fraction was checked (Graphs 6 - 8).
4. Each fraction was ashed in a muffle furnace and the residue was qualitatively checked.

When the Calgon technique was used on our in-service admixture, we obtained the following results:

1. The top fraction of the column contained mostly granular carbon plus a small amount of bone char with a high decolorization capacity.
2. The middle sections of the column did not separate enough to allow consistent evaluation.
3. The bottom fractions contained nearly pure bone char similar to the bottom fraction obtained when using pure bone char alone - in particle size, bulk density, percent decolorization efficiency and carbon content.

From our results, it appeared that if we used only the top fraction to represent the granular carbon, the bottom fractions to represent bone char, and neglected the intermediate zone, we could obtain a limited, but useful picture of what was occurring within the process.

While the possibility of impurities still existed, the method was the most workable for us of any found so far. The apparatus was readily available, set up was simple and samples were clean and of workable sizes. Although the method is not perfect, it can provide relative results to establish trends.

Furthermore, to be able to react quickly to changes occurring to the admixture in the refinery, a complete analysis has to be done in less time than a cistern cycle - usually from 5 to 7 days long. Therefore, the separations of composite cycle samples were needed within a week. Using the water fluidization bed method, analysis took only 24 hours working time, from start to finish.

Although the method gave us at best a limited picture of the admixture, it was adopted. Minor changes were made to reflect sampling techniques and the combining of multiple separated fractions. Specifically, we decided to use a composite sample consisting of one complete round of 12 cisterns. Samples were collected as cisterns were being charged with kilned admixture. An equal sample volume of 600 cc. was collected from each cistern and combined to form a composite volume of 7200 cc. The

composite was reduced by riffing and recombining to a final volume of 3000 cc.

Our separating column was charged with 500 cc. of a composite sample, and water was injected upward through the column at a rate of 1.7 l/min. for exactly 30 min. The water was then shut off slowly and the bed was allowed to settle. The column was drained and the sample rinsed with three bed volumes of distilled water. A vacuum was applied to the bottom of the column to remove any excess water.

The column was then dismantled and the first 100 cc. fraction was scraped from the top (granular carbon fraction #1). Two fractions of 100 cc. each were removed from the bottom (bone char fraction #4 & #5). The separation procedure was repeated four times and all the fractions were then respectively recombined and dried overnight at 150 degrees C. Samples #1, #4, #5 and an unseparated composite are analyzed. Results are shown on Graph 9. From the results, we were able to observe the following:

1. No relative changes in the admixture's decolorization capabilities over time.
2. An increase in the percent decolorization when the granular carbon content increased.
3. A rapid decrease in the percent decolorization efficiency when the overall carbon content of the admixture is too high.
4. The effect on the char fractions of the rise of available oxygen combined with higher kiln temperatures.
5. The relative changes in the granular carbon and bone char admixture.

We have recently observed a gradual change in the coloration of the ashed bone char fraction. An increase of reddish residue may indicate a gradual increase of granular carbon in this fraction, further limiting our evaluation capabilities. Our studies have led us to believe that the older the admixture the more difficult it becomes to separate it. It is likely that, over time, this separation technique will no longer be effective and another method will become necessary. Redpath, however, will not reach that point. The company has decided not to continue using the admixture in its charring cisterns, and the laboratory will not investigate other separation techniques.

TABLE 1 INITIAL ATTEMPT TO SEPARATE ADMIXTURE USING SETTLING METHOD

MEDIA	DENSITY (20°C)	REACTION OF	
		NEW BONE CHAR	NEW CANESORB
ETHANOL (95%)	0.75 g/ml	100% SINK	100% SINK
WATER	0.998 g/ml	100% SINK	100% SINK
LIQ. SUCROSE (67.5 Brix)	1.331 g/ml	100% SINK	100% SINK
LIQ. INVERT (77.5 Brix)	1.390 g/ml	100% SINK	100% SINK

TABLE 2 SEPARATING ADMIXTURE USING SETTLING METHOD
(TATE & LYLE RESEARCH GROUP)

MEDIA	DENSITY (TEMP) (20°C)	REACTION OF	
		NEW BONE CHAR	NEW CANESORB
ZINC IODIDE	2.64 g/ml (18°C)	MOST SINKS SOME FLOATS	100% FLOATS
	2.44 g/ml	90% SINKS 10% FLOATS	100% FLOATS
	2.28 g/ml	95%+ SINKS FEW PARTICLES FLOATS	99% FLOATS
	2.20 g/ml	99% SINKS	98-99% FLOATS
	2.08 g/ml	99%+ SINKS	95-97% FLOATS
	2.00 g/ml	100% SINKS	70-80% FLOATS 20% SUNK SOME SUSPENDED

Optimum separation is achieved at solution densities of 2.1-2.2 g/ml.

The above results were obtained from Tate & Lyle Research Group Monthly Report, B.C. Goodacre & S. Slocombe.

TABLE 3 SEPARATION AND ANALYSIS OF BONE CHAR/GRANULAR CARBON
(TATE & LYLE RESEARCH GROUP)

TEST	NEW CANESORB	NEW BONE CHAR	ADMIXTURE (80/20)	SEPARATED CANESORB	SEPARATED BONE CHAR
BULK DENSITY (lb/c.ft)	28.1	42.8	39.4	28.5	42.4
CARBON CONTENT (%)	80.91	9.38	21.08	82.20	10.46
INSOLUBLE ASH (%)	5.79	1.44	1.73	5.60	1.26
PHOSPHATE (%)	1.75	33.18	27.78	2.23	32.12
SCREENING ANALYSIS ON No.	8	0.1	0.1	0.1	0.5
	9	0.1	11.3	0.1	13.9
	12	19.9	39.3	15.6	38.7
	16	41.2	32.9	40.5	30.8
	28	29.9	14.3	35.2	13.1
	35	6.5	1.6	6.7	1.6
	60	1.9	0.3	1.4	1.0
Thru' 60	0.4	0.9	0.2	0.4	0.3

The above separation was performed by Tate & Lyle Research Group (B.C. Goodacre & S. Slocombe) and the analysis done by G. Irvine of British Charcoals & MacDonalds 1984.

TABLE 4 ANALYSIS OF THE FRACTIONS AFTER SEPARATION BY THE AIR
FLUIDIZATION METHOD

FRACTIONS	BULK DENSITY (lb/c.ft)	DECOLOR. EFFICIENCY	CARBON CONTENT (% by WT)	SILICA CONTENT (% by WT)
TOP FRACTION ("Carbon")	44.47	89.92	39.26	1.68
BOTTOM FRACTION ("Bone Char")	60.57	86.60	17.41	1.88
PURE CANESORB	29.82	97.50	86.13	5.92
PURE BONE CHAR	67.29	72.79	9.37	1.60

TABLE 5 ANALYSIS OF FRACTIONS AFTER SEPARATION BY WATER
FLUIDIZATION METHOD

FRACTIONS	BULK DENSITY (lb/c.ft)	DECOLOR. EFFICIENCY	CARBON CONTENT (% WT)	SILICA CONTENT (% by WT)
TOP 1/3 FRACTION ("CANESORB")	56.95	88.33	43.74	1.56
BOTTOM 2/3 FRACTION ("BONE CHAR")	71.33	71.08	12.37	1.29
COMPOSITE	66.78	76.31	21.44	1.53

TABLE 6 ANALYSIS OF FRACTIONS OF PURE CANESORB FROM WATER FLUIDIZATION METHOD

SAMPLES	BULK DENSITY (lb/c.ft)	SCREEN SIZE (% RETAINED)				THRU
		30	40	50	60	
COMPOSITE	27.86	85.11	11.69	2.75	0.17	99.72 0.28
12"-14" (TOP)	22.27	47.53	33.63	13.52	1.62	96.58 3.42
10"-12"	25.10	85.09	11.80	1.67	0.51	99.07 0.93
8"-10"	24.98	87.40	10.04	1.10	0.52	99.06 0.94
6"-8"	25.94	91.60	5.80	1.22	0.38	99.00 1.00
4"-6"	27.71	95.72	1.99	1.06	0.43	99.20 0.80
2"-4"	28.96	94.84	2.27	1.36	0.72	99.19 0.81
0"-2" (BOTTOM)	33.71	85.01	11.05	3.73	0.14	99.93 0.07

TABLE 7 ANALYSIS OF FRACTIONS OF PURE BONE CHAR FROM WATER FLUIDIZATION METHOD
(CHAR IS FROM A STOCK CHAR SAMPLE)

SAMPLES	BULK DENSITY (lb/c.ft)	30	SCREEN SIZE (% RETAINED)			30-60	THRU
			40	50	60		
COMPOSITE	64.55	45.63	19.38	19.19	7.64	91.84	8.16
12"-14" (TOP)	59.75	3.00	9.43	21.80	22.88	57.11	42.89
10"-12"	60.42	3.28	17.18	46.54	22.21	89.21	10.79
8"-10"	61.66	21.81	35.24	34.60	6.45	98.10	91.90
6"-8"	61.65	59.19	26.78	12.22	1.32	99.51	0.49
4"-6"	61.06	61.29	24.40	11.68	1.70	99.07	0.93
2"-4"	61.60	75.22	17.72	5.37	1.46	99.77	0.23
0"-2" (BOTTOM)	64.90	82.39	11.84	4.78	0.75	99.76	0.24

TABLE 8 ANALYSIS OF FRACTIONS OF ADMIXTURE FROM WATER FLUIDIZATION METHOD

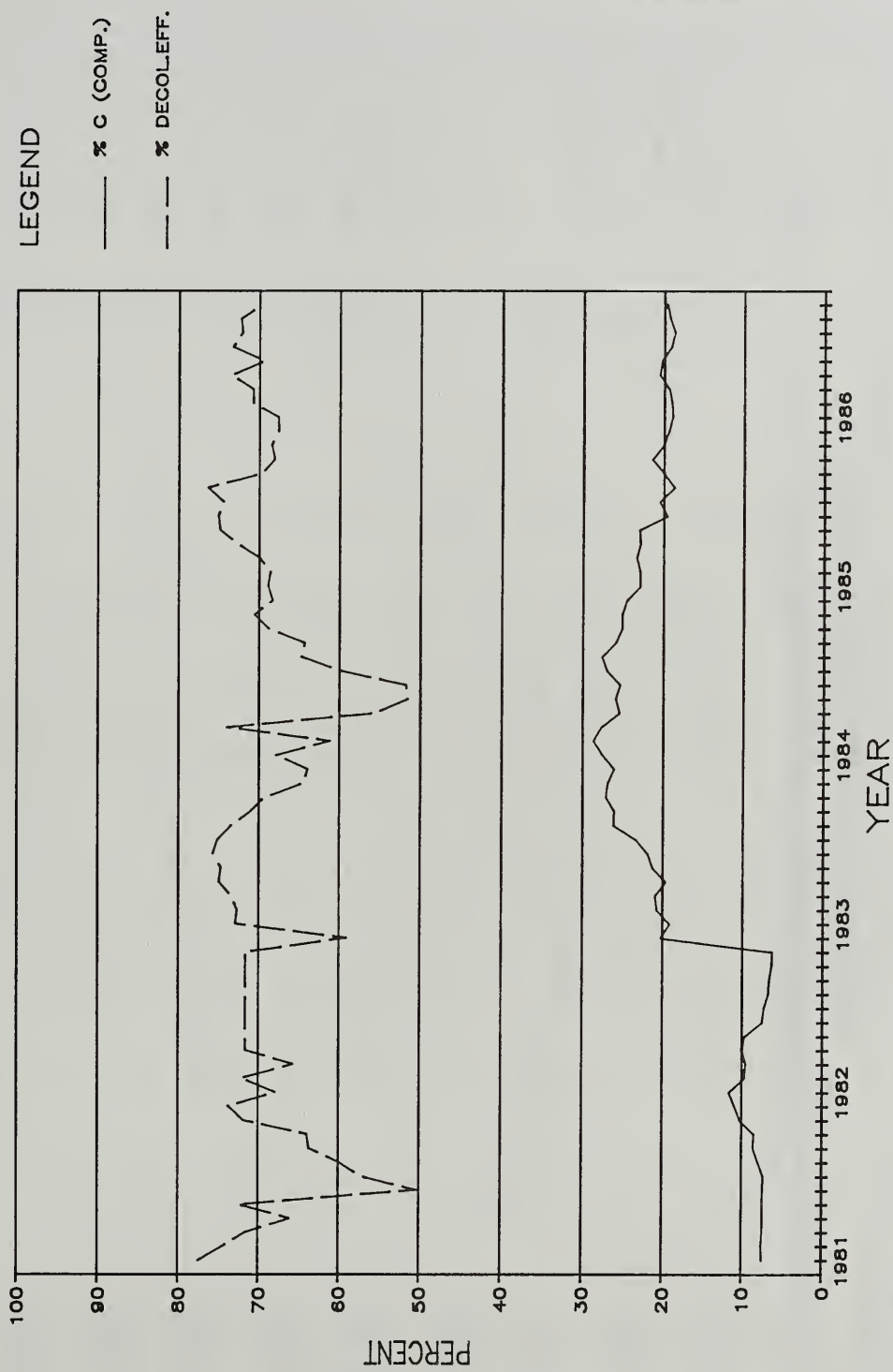
SAMPLES	BULK DENSITY (lb/c.ft)	DECOL. EFF. (%)	CARBON CONTENT (% WT)	SILICA CONTENT (% WT)	SCREEN SIZE (% RETAINED)					THRU
					30	40	50	60	30-60	
COMPOSITE	66.48	76.31	21.44	1.53	50.8	22.5	20.8	5.2	99.3	0.7
12"-14"(TOP)	49.66	83.77	25.15	1.31	54.5	11.6	18.2	11.0	95.3	4.7
10"-12"	62.53	81.79	24.69	1.17	29.6	19.1	38.8	9.8	97.3	2.7
8"-10"	68.93	77.15	20.27	1.20	19.2	31.8	41.0	6.8	98.7	1.3
6"-8"	70.41	72.85	10.07	1.26	24.3	40.0	32.7	2.9	99.9	0.2
4"-6"	71.40	66.23	7.56	1.34	54.1	35.1	10.3	0.5	99.9	0.1
2"-4"	68.79	55.30	5.94	1.67	81.7	16.5	1.8	0.1	100.0	0.0
0"-2" (BOTTOM)	63.57	54.30	5.83	1.22	97.1	2.4	0.5	0.0	100.0	0.0

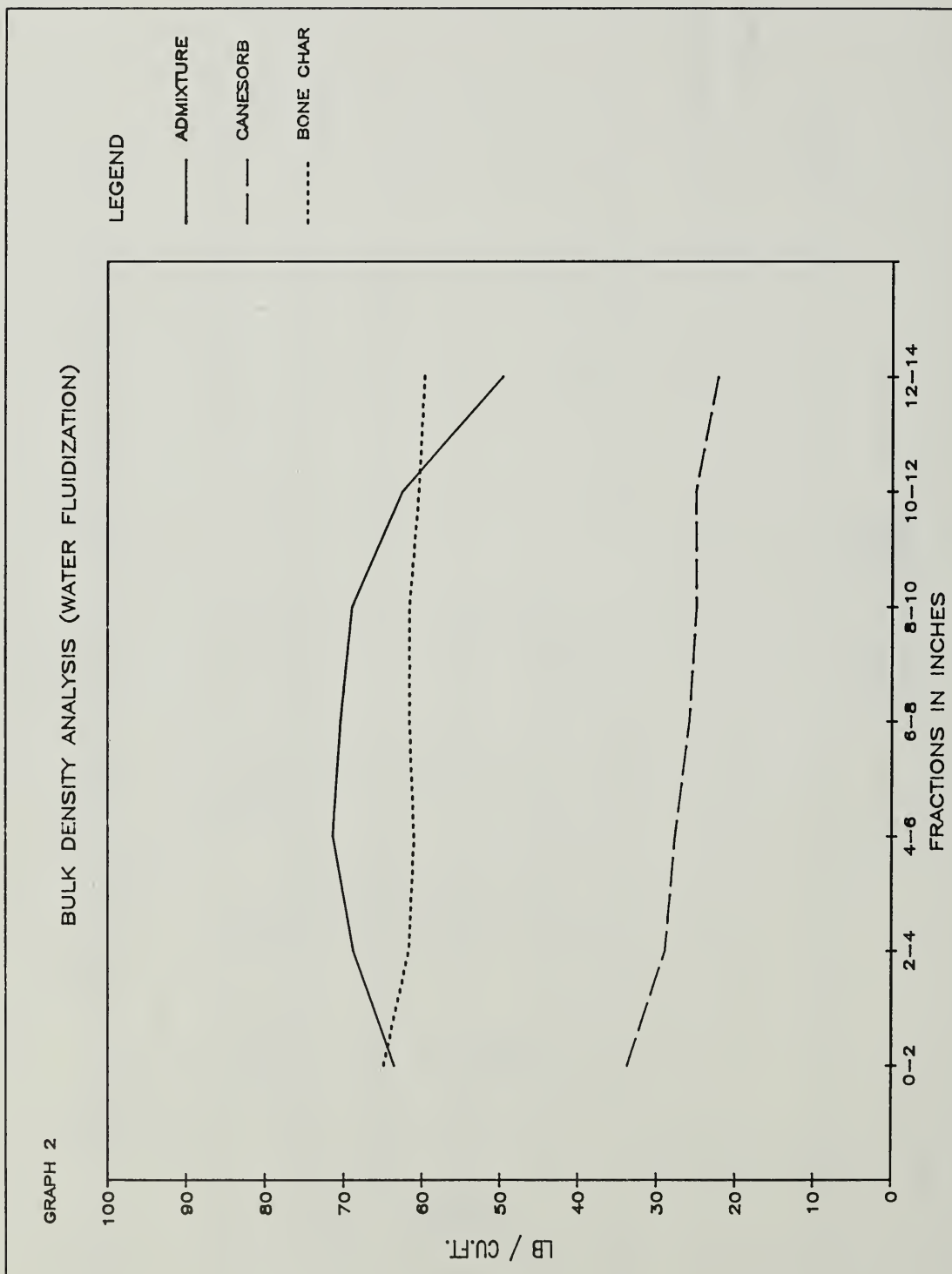
TABLE 9 ANALYSIS OF FRACTIONS OF 'HEAVY' ADMIXTURE FROM WATER FLUIDIZATION METHOD

SAMPLES	BULK DENSITY (lb/c.ft)	DECOL. EFF. (%)	CARBON CONTENT (% WT)	SILICA CONTENT (% WT)	SCREEN SIZE (% RETAINED)				THRU
					30	40	50	60	30-60
COMPOSITE	75.26	20.00	3.41	5.56	99.8	0.2	0.0	0.0	100.0
12"-14"(TOP)	73.10	18.98	3.93	5.85	99.4	0.4	0.0	0.0	99.8
10"-12"	73.97	18.07	3.19	4.77	99.6	0.4	0.1	0.0	100.0
8"-10"	74.58	22.71	3.19	4.51	99.7	0.3	0.0	0.0	100.0
6"-8"	76.43	22.34	3.11	5.11	99.6	0.4	0.0	0.0	100.0
4"-6"	76.01	20.15	2.79	4.88	99.8	0.2	0.0	0.0	100.0
0"-4" (BOTTOM)	75.66	23.44	2.58	5.72	99.9	0.1	0.0	0.0	100.0

GRAPH 1

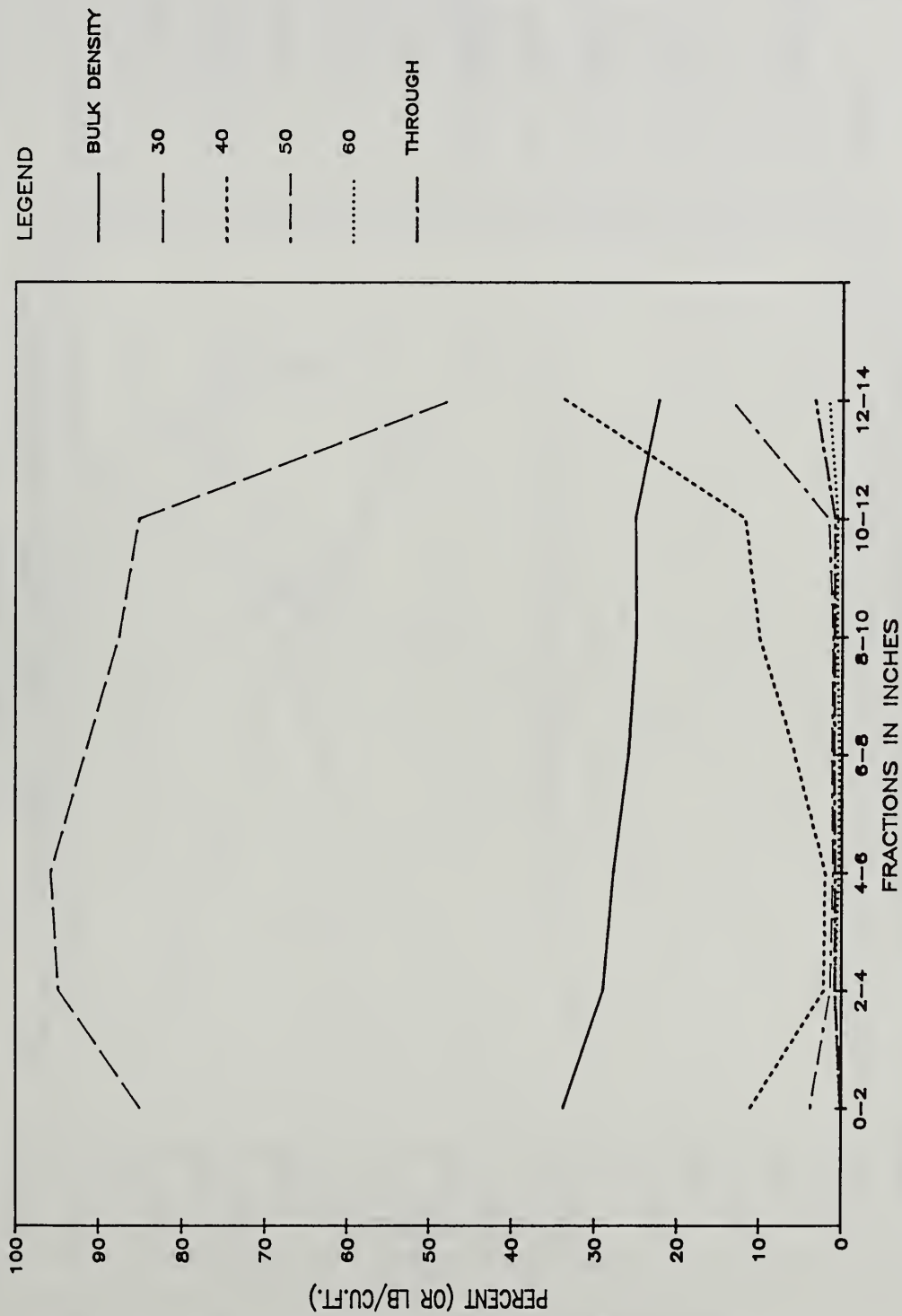
REDPATH SUGARS
DECOLORIZATION PERFORMANCE ANALYSIS
(1981 - 1986)

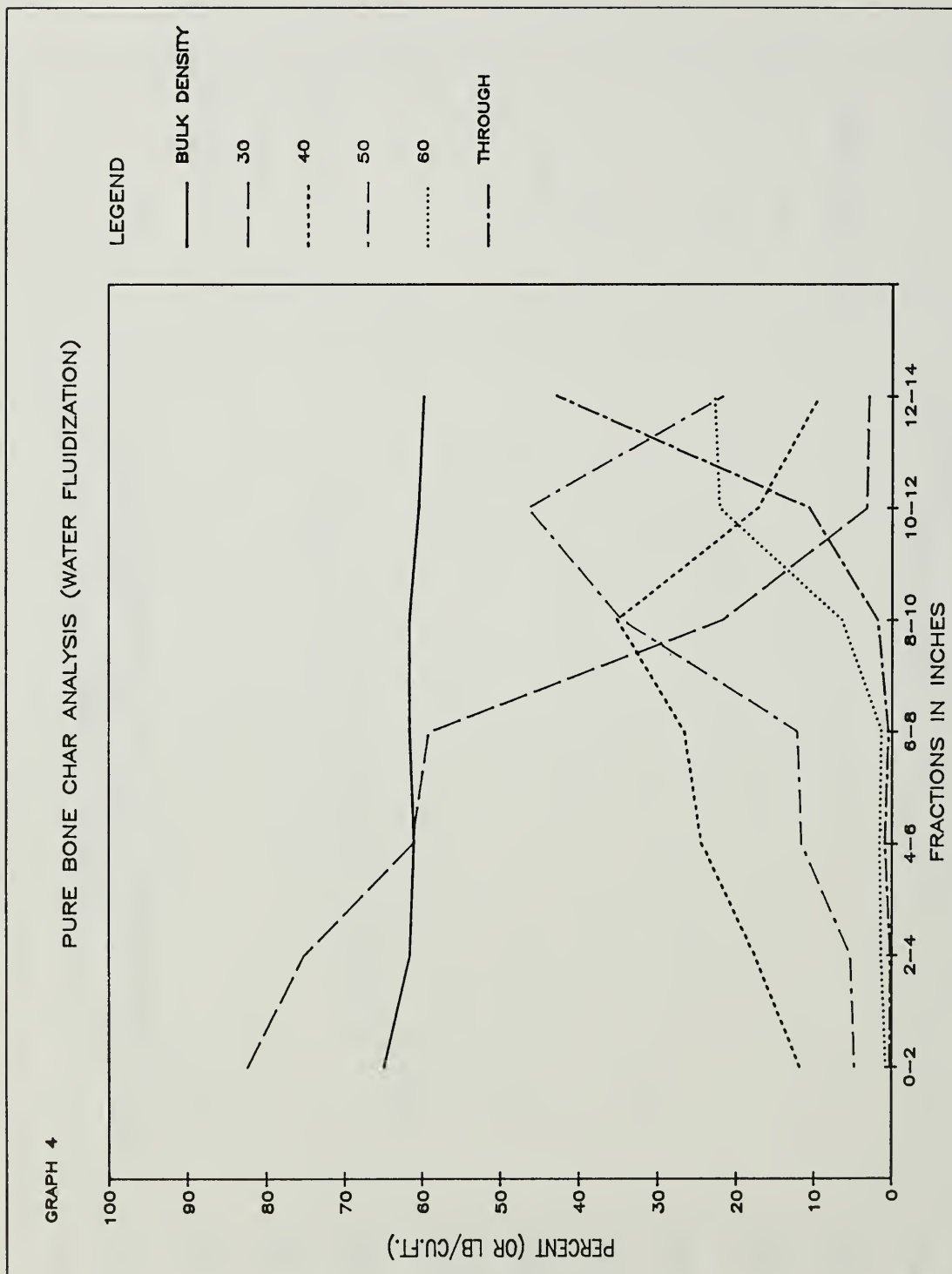


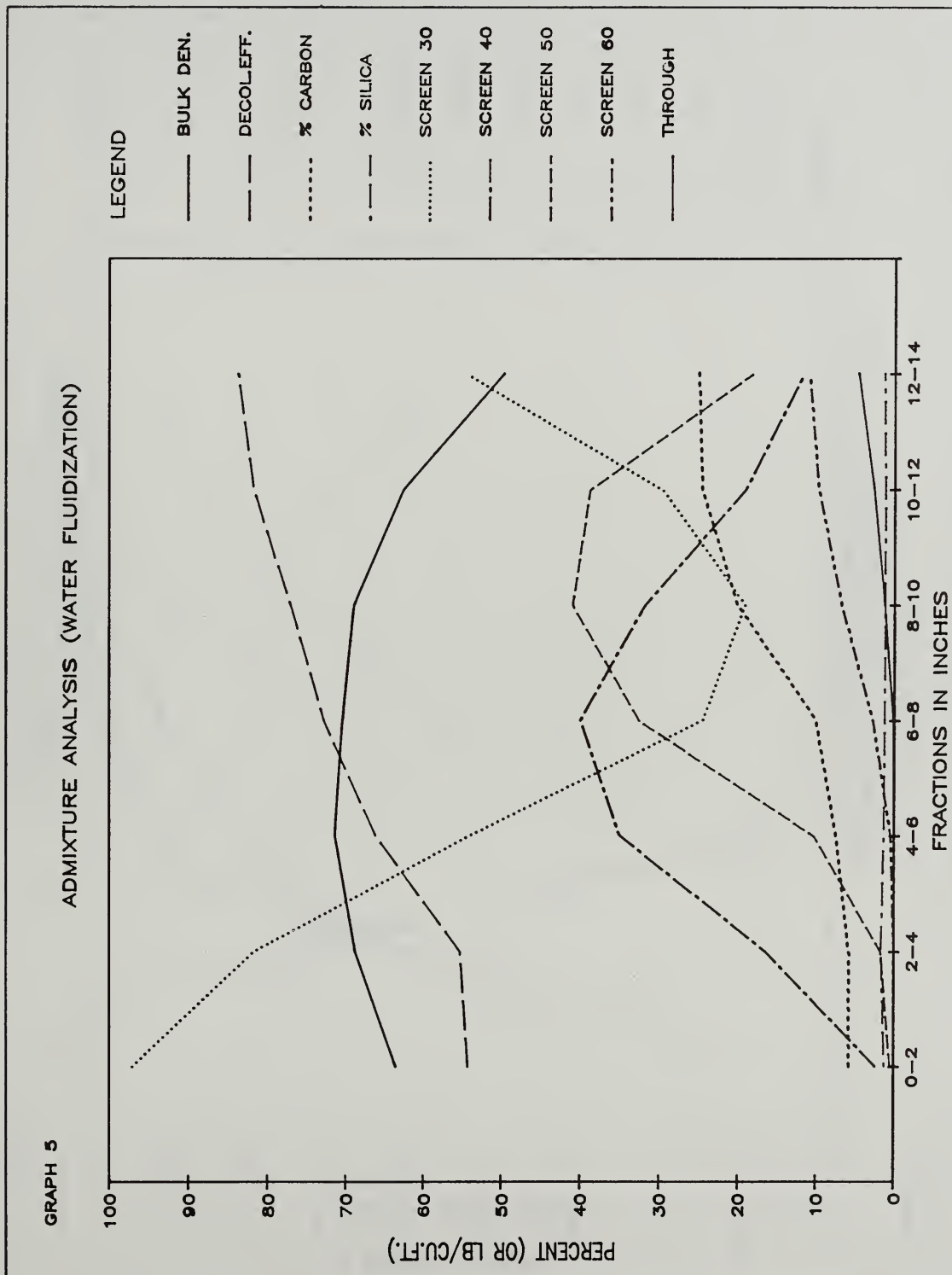


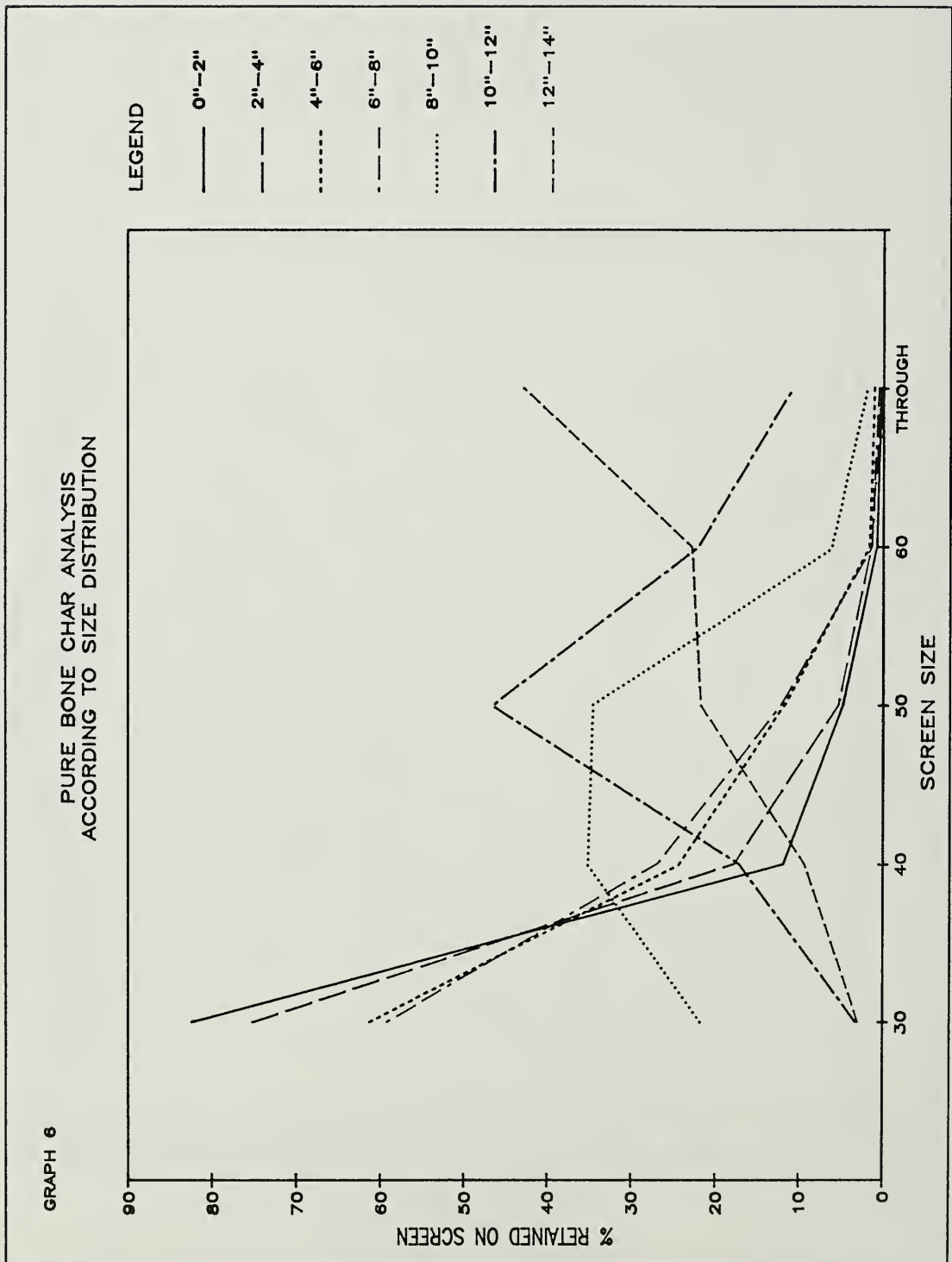
GRAPH 3

PURE CANESORB ANALYSIS (WATER FLUIDIZATION)



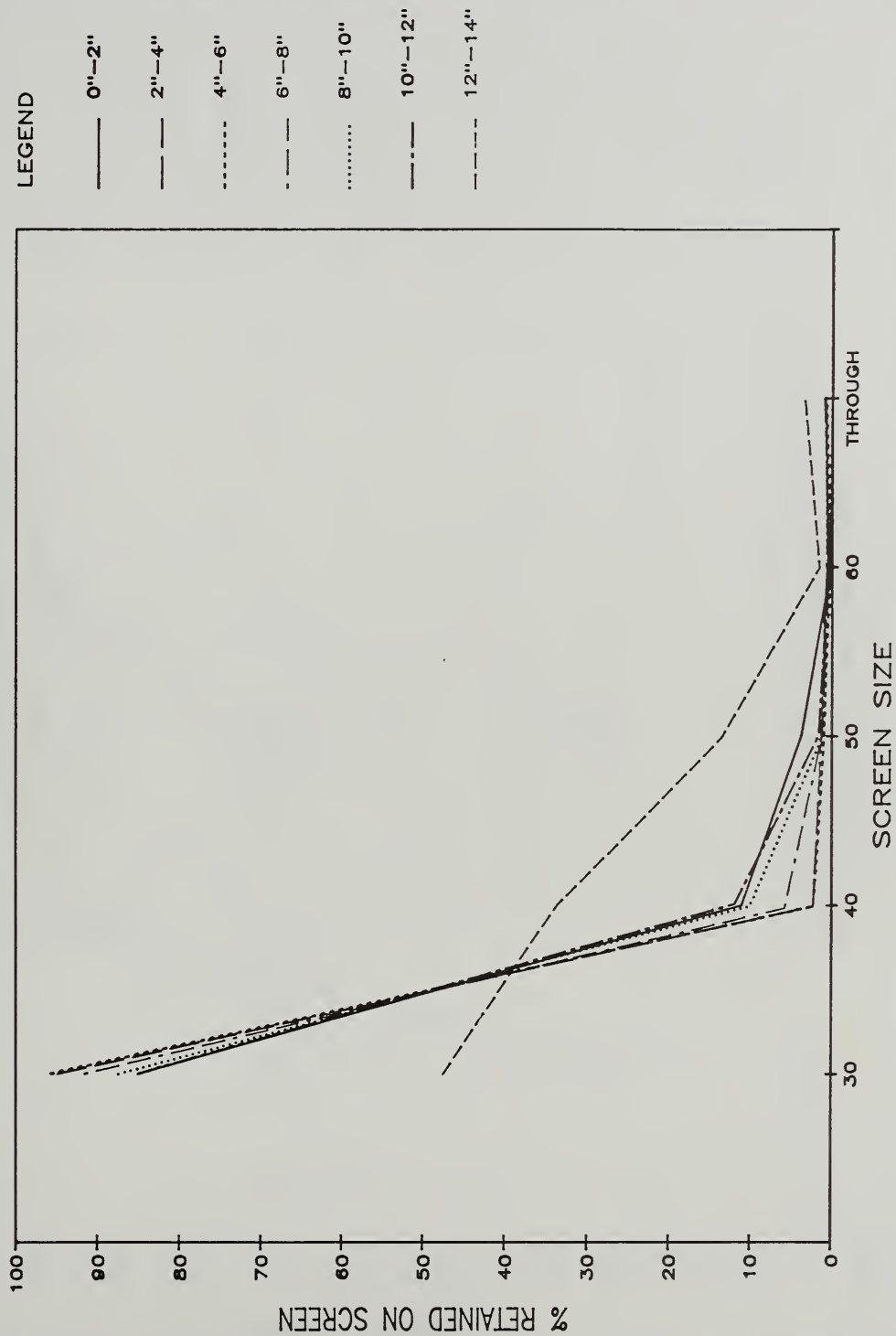






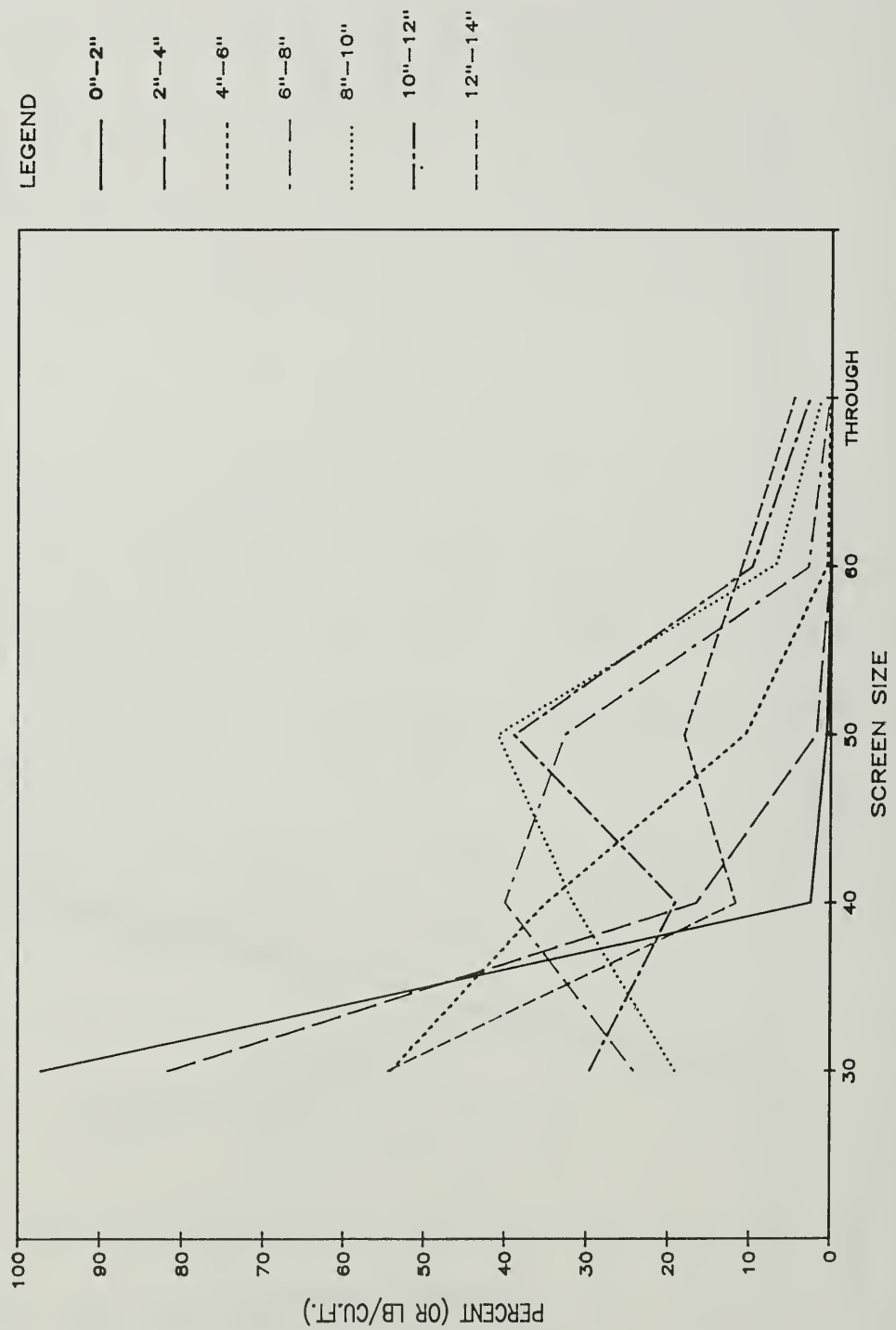
GRAPH 7

PURE CANESORB ANALYSIS
ACCORDING TO SIZE DISTRIBUTION



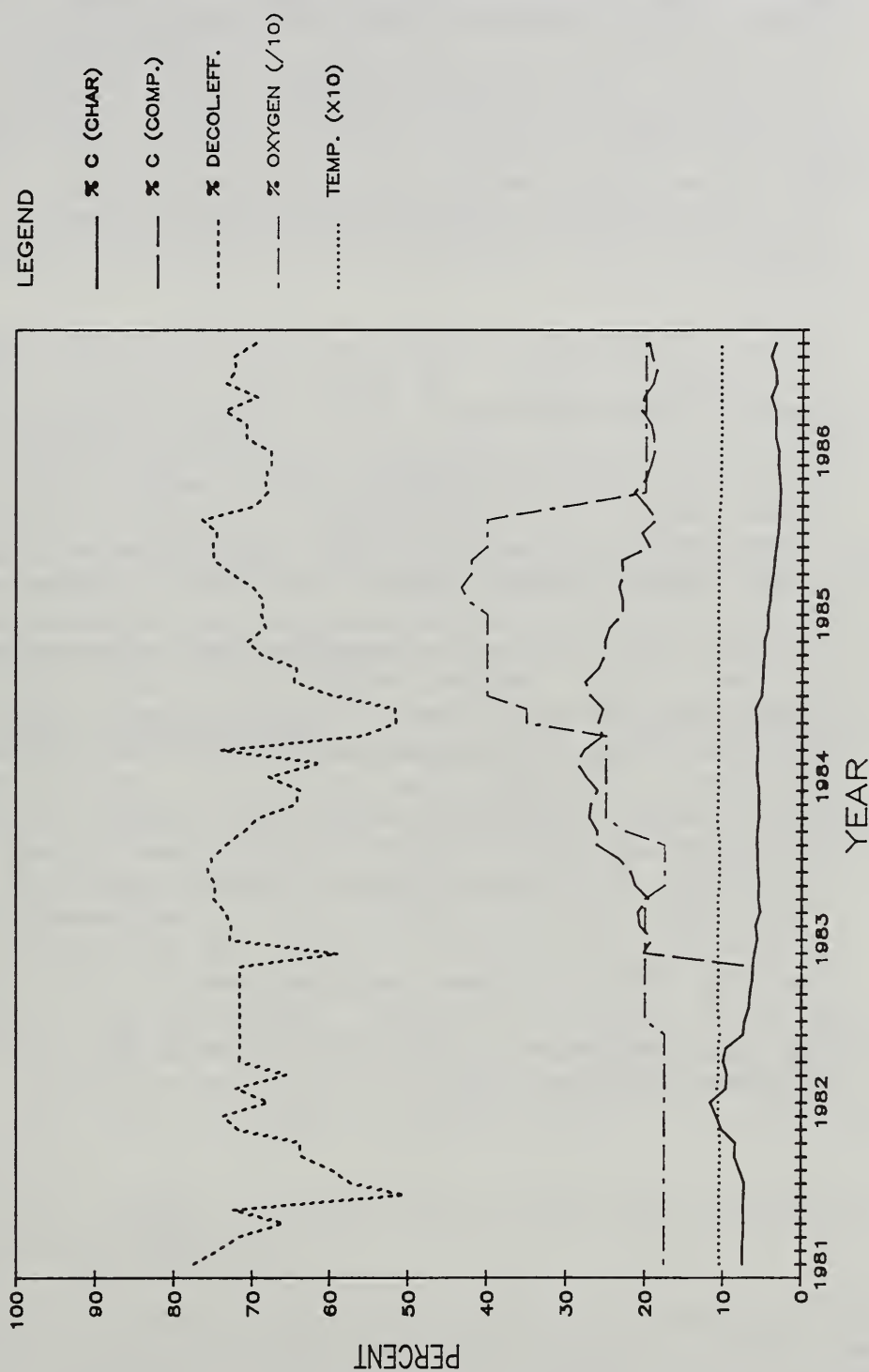
GRAPH 8

ADMIXTURE ANALYSIS (WATER FLUIDIZATION)
ACCORDING TO SIZE DISTRIBUTION



Graph 9

REDPATH SUGARS LTD CHAR HOUSE PERFORMANCE ANALYSIS (1981 - 1986)



APPENDIX

SEPARATION TECHNIQUE FOR INDEPENDENT ANALYSIS OF BONE CHAR AND GRANULAR CARBON - CALGON METHOD

1. Prepare a representative sample of granular carbon/bone char by riffing and recombining to a final sample volume of 500 cc. For composite analysis, equal volumes of each aliquot should be mixed to obtain a 500 cc composite.
2. Place the riffled sample in a Pyrex glass tube (2" i.d. x 18" length) which is fitted with a Teflon stopcock and 100 mesh (U.S. Sieve) screens at the top and base of the column.
3. Connect the stopcock nozzle to a source of distilled water with plastic tubing. The water source should be capable of supplying approximately 2 liters/minute for 30 minutes.
4. Pass water upflow through the sample bed at a flow rate of approximately 1.7 liters/minute to achieve a fluidized bed expansion of approximately 50%.. Adjust the water flow accordingly to assure uniform fluidization through the bed.
5. Continue the fluidization procedure for approximately 30 minutes or until complete separation is indicated by visual inspection. Turn off the water supply slowly and allow the bed to settle. Drain excess water from the column.
6. The granular carbon (top) layer will occupy a volume of approximately 180 cc and the bone char will occupy a volume of 320 cc. To prevent cross contamination, remove the "top" 100 cc from the bed for analysis of granular carbon. Discard the "middle" 200 cc of the bed and retain the "bottom" 200 cc for bone char analysis.
7. Dry the adsorbents at 150 degrees Celsius for three hours prior to analysis.

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DISCUSSION

Ben Jones, Savannah Sugar - I have a question that recognizes the difficulty of the separation. Would you comment on the ability to quantify the components of the admixture?

Dewar - As I mentioned, we were only able to determine the relative change in amounts of the total carbon vs. the carbon on char. The separation technique was not good enough to allow us to determine the actual amount of the admixture.

Richard Riffer, C and H Sugar - I found that if you screen the mixture before the hydraulic separation, you get a very sharp separation of 99%. It is a very easy separation. The other modification I made to the Calgon procedure is that I increased the length of the column by twice so that you can use about a kilogram of material at once and get enough material for laboratory decolorization tests.

Dewar - We tried the pre-screening, but we did not think of increasing the column length. Based on the column we had been using, all the pre-screening did was increase the length of time of the test. We had to be able to do something fast because our turn-over was very fast--five days--so we decided not to go with the pre-screening.

Mark Whukowski, Amstar - Did you try, at any time, to add dark liquor onto your admixture?

Dewar - This was the ultimate goal of using the admixture, but unfortunately, we did not get the decolorization we thought we would get. Originally, we started with a cistern cycle of about 14 hours, and we were hoping to extend it to 16 hours, but the cycle was instead reduced from 14 to 12 hours and now we are running at a 10-hour cycle with the admixture. So, we did not have the opportunity to try the dark liquors as was originally intended.

INTEGRATING LABORATORY INSTRUMENTS IN THE PROCESS CONTROL LAB

J.C. Thompson and W.J. Frazee

Lantic Sugar Limited

INTRODUCTION

In the early 1980's Lantic Sugar, like many other refiners, realized that the key to remaining competitive in the market place lay in increasing productivity through the introduction of new technology. The challenge facing today's control laboratory is to reduce operating costs while maintaining the level of services required for efficient operation of the refinery.

BACKGROUND INFORMATION

In the past, the laboratory has met the ever-increasing demand for more timely operating information by expanding its facilities out onto the plant floor in an effort to bring the laboratory closer to the operating personnel. By 1982, there were three distinct facilities in operation.

- A main laboratory, located on the second floor of the Pan House, adjacent to the office area, operating on a day shift basis. Housing supervisory and technical personnel this lab was responsible for the more sophisticated testing procedures not required for immediate technical or quality control.

- A laboratory located on the fifth floor of the Pan House, operating on a 24 hour basis. Manned by one technician per shift this lab was primarily concerned with finished product quality assurance.

- A laboratory located on the sixth floor of the Filter House adjacent to the liquor gallery, operating on a 24 hour basis. Manned by two technicians per shift this lab was responsible for process control.

With the creation of the new process control lab in 1981 it became apparent that administrative problems involved with maintaining these three facilities were contributing to the overall inefficient operation of the department.

Problems associated with this move included:

- Increased operating costs as a result of maintaining duplicate equipment and consumable supplies.
- Poor communication between the Filter House side of the refinery and the Pan House side.
- Unequal distribution of workloads.
- Low employee morale.

Time studies indicated that:

- 16% of the available working time was spent collecting samples.
- 11% was spent in calculating results and communicating these results to operating personnel.
- Only 52% of the time was spent in the actual performance of test procedures.

Methods and procedures used in the process control lab also had a negative impact on manning requirements in the day lab. For simplicity and speed apparent purity or pol percent spindle brix was used for process control purposes; however, polarizations on incoming and outgoing materials, as measured by the day staff, were done using a normal weight (26g of sample diluted to 100ml) clarified with wet lead. Purities were calculated using the refractometric dry solids of the original sample. In order that operating records conform, it was necessary to retest samples, composited on a 24 hour basis, using the normal weight method. In addition, the day lab was responsible for summarizing process information and compiling a number of periodic reports.

It was proposed that, to reduce administrative costs, the laboratories be consolidated into one facility. Access to the laboratory would be provided by a pneumatic sample delivery system with inlets at all major sampling points, thus eliminating the non-productive travelling time. In order to make real productivity gains, it would be necessary to automate many of the test procedures currently performed by the process control lab. The system designed must meet the following criterion:

- 1) The system must be able to handle the entire range of sample types currently tested with a minimum of sample preparation.
- 2) Processing time must be less than 4 minutes per sample.
- 3) Purity determinations must be made without prior clarification with lead subacetate and the results must be in agreement with the normal weight method used in the day lab.
- 4) Provide immediate feedback to operating personnel and produce periodic summary reports.

Furthermore, the entire project must have a pay-back period of less than three years.

SYSTEM DESIGN

The control lab is primarily concerned with the measurement of a number of physical properties such as, refractive index, light attenuation, polarization and conductance. These measurements are then used to calculate a variety of control parameters. With the advent of commercially available equipment designed to translate the data output of the measuring device into computer compatible input, it is possible to link these devices to a host computer to provide an integrated system capable of the simultaneous determination of a variety of control parameters. In 1977, Kettlewell and Maylott described two such systems, one for color and one for purity determinations, in use at C&H Sugar (1). The system in use at the Saint John refinery is designed for the simultaneous measurement of pH, color, refractometric purity and conductivity ash and the dissemination of results to the plant floor.

The measuring train consists of the following analytical instrumentation. A block diagram is provided in figure (1).

- 1) A critical angle refractometer, equipped with a water-cooled flow-through sample chamber. The measuring range is between 0 and 50 brix with a resolution of 0.01 brix. Analog output is from 0 to 2.5 volts.
- 2) A polarimeter fitted with a water-cooled flow-through cell with a 5mm pathlength. Operating on the principle of magneto-optical compensation the polarimeter has an effective measuring range of $\pm 2.3^\circ$ at 589nm with a resolution of 0.001° . Analog output is ± 2.0 volts.

This type of polarimeter uses faraday rotators as both modulator and compensator. The optical rotation of the solution in the polarimeter cell is compensated by an equal but contrary rotation of a glass rod forming the core of a D.C.-powered solenoid (faraday cell). The compensator angle of rotation is proportional to the solenoid current.

The advantage of this design, for this particular application, is the high degree of angular resolution available which allows for the use of short cell lengths. Since light transmittance varies exponentially in relation to pathlength, while polarization varies linearly, it is possible to obtain accurate measurements on highly colored solutions without prior clarification. Thus, a solution with a polarization of 34.63° (100°S) and a transmittance of 0.0001% in a 20cm cell will have a polarization of 0.866° and a transmittance greater than 71% in a 5mm cell. The minimum level of transmittance in the polarimeter cell is 0.1% (absorbance 3).

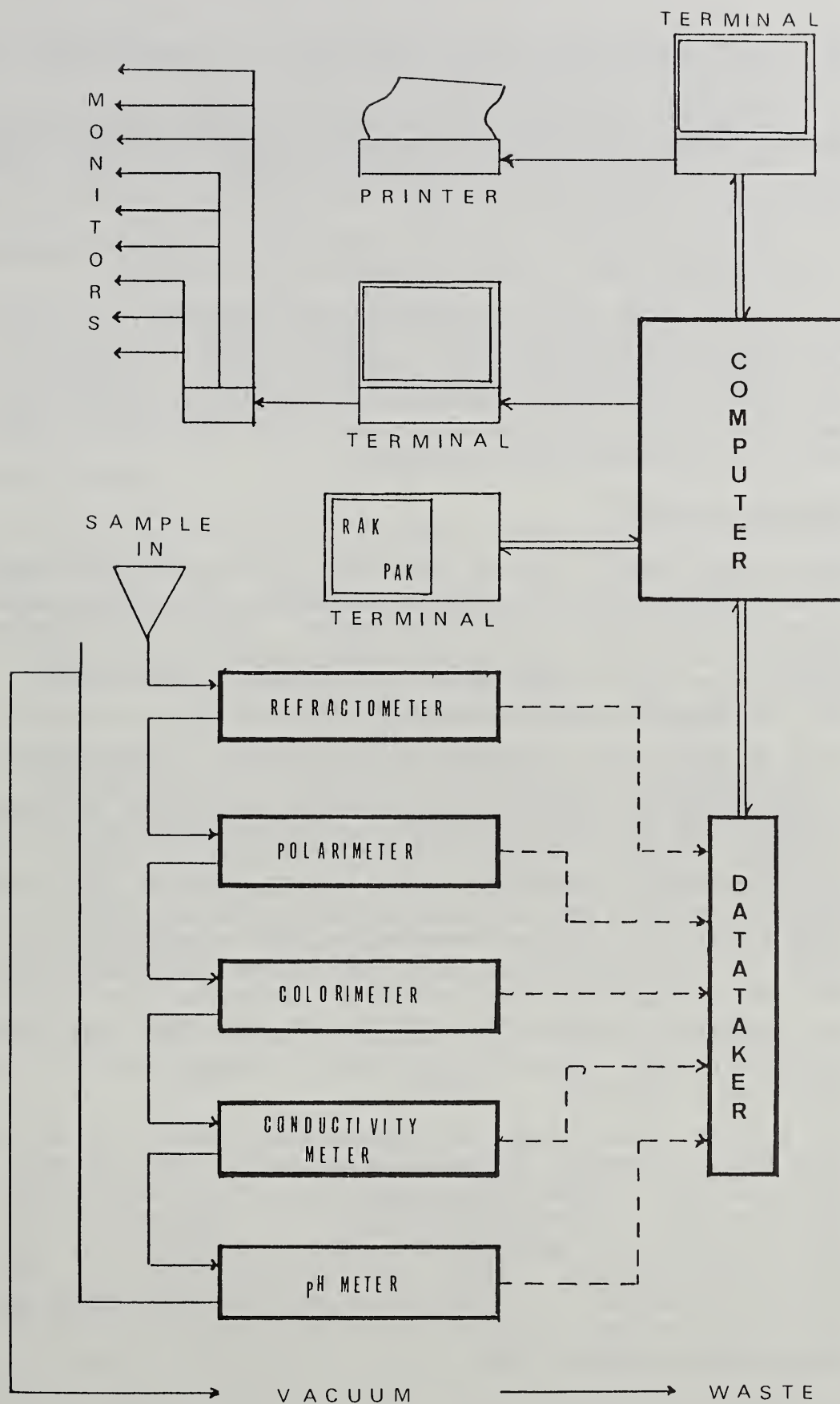


Figure (1): System Schematic

3) A spectrophotometer set at 420nm and fitted with a 1cm flow-through cell. Analog output is ± 1.0 volts.

4) A conductivity meter with an "in-line" conductivity sensor, housing two stainless-steel electrodes, fitted in a flow-through cell. The measuring range is between 0 and 10 mmhos with an analog output from 0 to 5 volts.

5) A pH meter with an "in-line" combination electrode housed in a flow-through cell. Analog output is 0 to 1.4 volts.

The sensing cells of all the analytical instruments are joined with 3/16" I.D. Tygon tubing. A funnel is used to introduce the sample. The sample is moved with the aid of vacuum provided by an aspirator connected to an adjacent cold water tap. The refractometer and polarimeter cells are maintained at 20°C with a refrigerating bath/circulator.

COMPUTER INTERFACE

The voltage outputs from the individual instruments are linked to the computer via a microprocessor based, smart analog multiplexor unit referred to as the Datataker. Supervised from the computer, this unit converts the analog signals into a form suitable for input to the host. All communications to and from the Datataker are in standard ASCII format.

Up to 23 analog input channels can be utilized. Input lines to the appropriate channels are connected via a 25 pin I/O connector and the individual pins of these connectors are linked to input channels on an internal wire wrap patch panel.

The A/D converter autoranges over 3 decades to ± 2.5 volts. Resolution is 1:40,000 and accuracy is 0.15%. Voltage signals greater than 2.5 volts are measured by first attenuating the signal to the 2500mv range, using the internal attenuation network and then applying this voltage to the analog input channel.

The Datataker is controlled continually by the host computer via an RS 232 C link. Channels are scanned on demand and values returned for processing.

The operator communicates with the computer through a rack-mounted video display terminal, referred to as the Rac Pak, equipped with an RS 232 C communications port and a 28 key sealed membrane key pad. The program controlling the acquisition of data runs continually on this terminal. The operator uses the key pad to log in samples, initiate data acquisition or enter additional information. It is mounted above the bench surface and its design is ideally suited to the often sticky environment of the process control lab.

COMPUTER HARDWARE

The host computer is a 512 K RAM, Texas Instrument 373A mini-computer with a 39 megabyte hard disk drive and 14 megabyte tape backup. This is a multiuser system which can support up to seven individual work stations.

PERIPHERAL DEVICES

In addition to those devices already mentioned, there are three TI work stations, two line printers and a Lear Ziegler terminal with an inactive keyboard. The video output from this terminal is amplified, split and sent to seven production floor monitors.

SOFTWARE DESIGN

All software is written in T.I. Basic. The function of the program is fourfold:

- 1) To accept raw data from the Datataker and the keypad of the Rac Pak.
- 2) To process the raw data into usable form.
- 3) To print results to the printer and display terminal.
- 4) To accumulate the data in a database.

The program is coded in 4 logical sections which correspond to 4 separate preprogrammed visual display screens for the Rac Pak. These are used to guide the operator through the entire sequence of sample log in, manual data entry, instrument calibration and analysis. Navigation from one screen to another is accomplished in one keystroke by pressing any one of the four function keys on the bottom row of the keypad as shown in table (1).

TABLE (1) - KEYS USED FOR NAVIGATING THROUGH THE PROGRAM

KEY	SCREEN
E	Opening Screen
I	Log-in Screen
II	Brix Screen
III	Zeroing Screen
IV	Analyser Screen

SYSTEM OPERATION

To run a sample, or series of samples, the operator calls up the "log-in" screen. Using the cursor control he selects from one of 50 main sample types displayed. If the computer requires more information, it responds with a query in the message block.

The operator keys in any additional information or uses the cursor control again to select from a series of sub-sample types displayed. The computer determines which tests are required for that sample and returns a number for sample identification.

If the brix of the as received sample is required, the operator determines this manually using an abbe refractometer. He then calls up the "brix" screen and the computer displays all samples currently awaiting test results. The first sample in the queue can be called up by pressing the enter key or, if the samples are being run out of turn, by keying in the actual sample I.D. number. The operator then enters the measured brix, the value is placed in temporary storage and the sample is removed from the list.

To determine the color, refractometric purity, pH and/or conductivity ash, the operator dilutes a portion of the sample in distilled water. In theory, any dilution which will not exceed the measuring range of any of the instruments is permitted; however, in order to stay within the accuracy limits of many of the empirical calculations involved, high purity intermediates requiring a pH, color and conductivity ash are diluted to a range between 18 and 22 brix. Darker samples requiring purities are measured between 6 and 12 brix.

The zero values for the instruments are determined by flushing the cells with distilled water, calling up the "zero" screen and, when the voltage readings have stabilized, pressing enter. The values are stored and used as baseline values for the proceeding samples.

The operator calls up the "analyser" screen and the computer displays the list of samples awaiting analysis. Two hundred to 250 ml of sample are poured into the funnel, drawn through the measuring cells and the appropriate sample number entered. The computer scans the Datataker at 10 second intervals, calculates the test results required for the particular sample and displays the values on the Rac Pak. Each new value is compared to the value from the preceding scan until no significant difference is detected. Once stability has been achieved the values are compared to a set of reference values for that sample type. If the values are not within the expected range, an error message is returned. After the operator has assured himself that the results are indeed correct, he can override the check feature, in which case the results will be printed to the plant monitors with highlights to indicate a non-standard condition. If the results do lie within the expected range, or the check function is overridden, the values are placed in temporary storage and the sample number is removed from the list. The analyser is now ready for the next sample.

Each time the results are placed in temporary storage, from either the "brix" screen or "analyser" screen, the computer checks to see if all the required tests have been performed for that sample type. Only when all tests are complete, will the computer print the results. Results are printed, to both the line printer and display terminal, as a single line of data containing the sample code, log-in time and appropriate test results. In addition, the time elapsed between log-in and printing is recorded on the line printer. Up to 24 lines can be displayed on the video monitors on a first up-first out basis.

The results are also stored in two database systems located on the hard disk. The first database is a first in-first out file which holds the last 12 results for any particular sample type. This file can be accessed from any of the T.I. work station terminals where the user can call up the last 12 results for any sample type for analysis. The user can also display the most current results for all sample types in a tabular form.

The second database is a series of running average files used to print period reports. Results for each sample type are stored as average values from the time the file was last erased. There are three files, one for the shift report, one for the daily report and one for the weekly report. As a report is printed the data is dumped up to the next level and the reported file erased. Weekly averages are stored as separate lines of data on the disk and printed individually on a monthly basis. Each report lists the sample type, the number of samples processed during the period and the average values for each test. Figure (2) shows a portion of a daily report.

PERFORMANCE CHARACTERISTICS

The system has been in operation for more than six months with no major hardware problems. Between 200 and 300 samples are processed in a 24-hour period with an average analysis time of 2.5 minutes per sample.

Table (2) lists average purities determined on 12 samples. The standard deviation ranged from 0.00 purity units to 0.46 purity units and averaged 0.20 purity units.

These refractometric purities are in good agreement with those determined by manual methods using a normal solution (26g of sample diluted to 100ml) clarified with lead subacetate (see table (3)). As these results are used solely for process control, no correlation with "true" sucrose purities has been attempted.

L A N T I C S U G A R

Daily Report -- 10/01/86 -- 1:27pm
For 09/30/86

WETLAB RESULTS

*****	BRIX	pH	PURITY	COLOR	TURB	ASH	
WSL COLOR	67.97	6.96		1331		0.16	12
WSL C/T				893	398		2
CWSL COLOR	66.58	6.94		567		0.15	11
CWSL C/T				499	41		2
REM TO 85TK	65.83	7.17		188		0.18	18
REM TO MELT HOUSE							**
85TK AND 86TK COLOR	66.95	7.15		559		0.17	11
85TK AND 86TK C/T				535	63		2
NEW LIQUOR	66.52						9
FINE LIQUOR COLOR	72.52	8.02		117		0.14	11
FINE LIQUOR C/T				110	8		2
1ST SYR	71.88	7.52		383		0.39	6
2ND SYR	73.37	7.08	98.12	890		0.96	6
3RD SYR	72.22	6.62	95.90	1976		1.75	6
RSL	64.76	6.80	97.92	6466		0.88	12
CRSL	62.78	7.23	98.57	4538		0.89	12
SSL	68.49	6.85	88.04	13181		4.01	11
CSSL	66.45	7.11	88.42	11080		4.06	11
Y. LIQUOR	64.51	7.31	88.82	4420		3.86	18
ROTO WATER	16.25	7.05					4
AUTO DOSING WATER	12.78	6.20		2371		1.02	5
LIGHT CHAR WATER	45.30	8.70					1
HEAVY CHAR WATER	72.10	7.10					1
AFFINATION	74.45	7.05	85.57			4.12	6

Figure (2): Portion of Daily Report

Table 2: Refractometric Purity and Conductivity Ash

Sample	No. of Runs	Purity		Conductivity Ash	
		Average	Standard Deviation	Average	% Deviation
set#1	4	99.6	0.10	0.12	0.0
set#2	4	99.7	0.20	0.14	4.4
set#3	4	99.6	0.00	0.13	3.8
set#4	4	99.4	0.15	0.35	2.9
set#5	4	97.7	0.08	0.98	1.0
set#6	4	95.4	0.15	1.64	1.2
set#7	4	89.8	0.25	2.81	0.4
set#8	4	87.7	0.24	3.17	0.7
set#9	4	86.1	0.22	3.62	1.1
set#10	4	73.5	0.19	6.91	0.7
set#11	4	65.9	0.46	8.68	0.3
set#12	5	57.2	0.39	11.0	1.1

Table 3: Comparison of Manual and Automated Purities

Sample	Manual Purity	Automated Purity
set#7	89.41	89.82
set#10	73.52	73.48
set#11	65.65	65.92
set#12	56.73	57.24

Table 4: Color Performance Data

Sample	No. of Runs	Color(I.C.U.)	
		Average	% Deviation
set#1	4	121	0.67
set#2	4	534	0.81
set#3	4	1492	0.88
set#4	4	400	0.78
set#5	4	1235	0.03
set#6	4	2440	0.38
set#7	4	5261	0.58
set#8	4	17732	0.48
set#9	4	14452	0.06
set#11	4	18837	0.26

Table (2) also lists the average conductivity ash determined on these 12 samples. The standard deviation, reported as a percentage of the average value, ranged from 0.0% to 4.4% and averaged 1.4% of the average ash.

Color can be determined accurately, within the normal concentration range of 6 to 12 brix, up to a maximum of 20,000 ICU after which the linearity of the spectrophotometer output is exceeded. If precise purity or ash values are not required, then colors in excess of 20,000 can be measured by reducing the brix of the sample solution in order to maintain a transmittance above 1% at 420nm. Fortunately, the bulk of our color control work is concerned with colors less than 20,000 ICU.

Table (4) lists average colors for 10 samples. The standard deviation, expressed as a % of the average, ranged from 0.03% to 0.88% and averaged 0.49%.

FINISHED PRODUCT QUALITY EVALUATION

No attempts have been made, as yet, to integrate any of the instruments used for finished product evaluation. Instead, the results are entered into the computer, as they become available, using a menu driven program running on one of the T.I. workstation terminals. The technician calls up the appropriate product type to the screen and then enters the raw data in response to a series of input requests by the computer. The computer performs any calculations which may be required and prints the results as a line of data to the printer and the video terminal. The results are also accumulated in the database and included in the periodical reports.

Many of the test results from the day shift laboratory are also entered into the database in much the same manner as the finished product results. This data entry system is still being expanded with the ultimate goal of computerizing all laboratory records.

CONCLUSIONS

The installation of the sample delivery system and the automation of the process control lab has permitted a reduction in manning requirements from three to two technicians per eight-hour shift, as well as a reduction in day shift manning from four to three technicians, for a total reduction of four positions. In addition, it has provided operating personnel with earlier analysis and more accurate results and, as a result, has had a positive impact on both refinery performance and product quality. The project has met or exceeded all the design criteria referred to previously and the pay-back period has been calculated at 14 months.

REFERENCE

- 1) Kettlewell S.F., and A.O. Maylott
1977. Laboratory Automation at C&H Sugar Company.
Sugar Ind. Technol., 36, 159-165.

DISCUSSION

A. Bartolo, Imperial Sugar - What qualifications do you require for your technicians that work in the process lab, and how have they adapted to the changes that you have introduced?

Thompson - The technicians in the process control lab. are hourly paid employees who are part of the collective bargaining unit, so the main criterion is plant seniority in hiring these people. The minimum requirement is grade 12 or high school equivalent. Their response to the system has been very enthusiastic. We have had no problem in training because it is a menu-driven program. It is very easy to pick up and use. The technicians actually feel less pressure and enjoy working the system very much.

Toshio Moritsugu, H.S.P.A. - What do you consider a good c.v. and a poor c.v.?

Thompson - We try for a c.v. of about 25%.

RECENT OBSERVATIONS ON SUGAR COLORANTS IN CANE SUGAR REFINERIES

Margaret A. Clarke and Rebeca S. Blanco

Sugar Processing Research, Inc.

INTRODUCTION

This report presents results on recent studies related to three areas of sugar colorants. The major area of discussion is the recycling of colorant in the melt house of the sugar refinery: how much colorant is recycled, in both remelt materials and melt sweet water, of what types are these colorant materials, and the relative amounts of colorant recycled and generated in the melter. Initial results on separation of very high molecular weight colorant in raw sugar are reported, and a comparison of raw sugar color measurement at pH 7 and pH 8.5 is presented.

In continuing studies at S.P.R.I. on the nature of colorant in raw sugar (Clarke et al., 1984) and the removal of colorant types by refining processes (Clarke et al., 1985), a study was begun, in cooperation with Alan M. James, of S.K.I.L., on recycling of colorant in the melter. Initial results were reported at the Sugar Industry Technologists' Conference in May, 1986 (James et al., 1986).

It is a basic premise of sugar refining, held as a firm rule by Oliver Lyle and George Meade, that at each step of refinery operations non-sugars are separated from sugar and should not be added back. Most processes are operated according to this principle, but because sweet waters contain sucrose, as well as non-sugars, and this sucrose must be recovered, it is necessary to add back these non-sugars. This is usually done by using the sweet waters as melter water.

In the 1985 S.P.R.I. study of colorant across refineries, data, shown in Table 1, from several plants, indicated an increase in total colorant from washed raw sugar to clarified liquor, instead of the decrease in color that is expected across each stage in process. In all analytical surveys of plants in operation, difficulties in coordination and timing of sampling can produce artifacts, but this increase was observed with a frequency sufficient to justify further investigation.

Table 1.--Color data from study across refineries (Clarke et al., 1985)

	Refinery A Colors, 420 Set b	Refinery C pH 7 Set a	Refinery D Set b
Washed raw sugar	402	907	1110
Melt liquor	987	2032	1378
Color addition %	143	124	24
Clarified liquor	764	1378	1143
Color reduction %	22	32	17
Color change % (Washed raw-clarified)	+90	+52	+3
% Color load in melt liquor from recycle	58.9	55.4	24.4

No information on melt liquor composition.

Refiners A & C phosphatation.

Refinery D carbonatation.

The study on colorant levels before and after the raw sugar melter was then organized; whole color, at 420 nm, pH 7, was measured and levels of phenolics and amino nitrogen were measured by tests described in the earlier papers.

Results shown in Tables 2 and 3 from two refineries, one, A, on phosphatation/bone char, the other, B, on carbonatation/bone char indicated that there is a considerable degree of color recycled from various refinery sweet water streams back into the melter. Refinery A, which did not add remelt to the melt stream, showed an average 62% increase in color in melt liquor over the color of washed raw sugar, and indicated that some 38% of this color came from material recycled to the melter, i.e. from melt sweet water, plus color regenerated by heat in the melter. Refineries A and B in this study are not the same as those referred to in Table 1.

Table 2.--Refinery A - phosphatation/char (James et al., 1986)

	Brix	Color		I.V.	Phenolics ppm	Amino N ppm
		420 pH 7	560 pH 7			
Raw sugar		2927	542	3.33	56	88
Washed raw sugar		811	157	4.08	14	24
Washed sugar liquor	65.2	1319	284	3.15	23	41
Dilution water	15.0	2532	547	2.47	400	438
% change from W.R. sugar to melt liquor		62.6	80.1		64.3	70.8
% load in melt liquor from recycle		38.5	44.7		39.1	41.5

Average of 12 sets of samples.

Most of the recycled color has been at high temperature for some time, and so colorant in the melt sweet water may be expected to contain a high proportion of polymerized melanin-type colorant, as indicated by the high level of response to both phenolic and amino nitrogen tests. Molecular weight distribution analyses on the recycled colorant are not yet available.

Average values for samples from the melt house of refinery B are shown in Table 3. Because this refinery adds some 10% of the solids to the melter as remelt, the increase in color from washed raw to melt liquor is much higher than in the earlier case, as is the percent color in melt liquor that derives from sources other than washed raw sugar.

Table 3.--Refinery B - carbonatation/char

	Brix	Color 420 pH 7	Color 560 pH 7	I.V.	Phenolics ppm	Amino N ppm
Raw sugar		3390	668	2.49	58	57
Washed raw sugar		902	178	3.60	15	15
Remelt	56.8	11134	2358	1.77	162	111
Dilution water	6.3	4336	1216	1.90	1211	1842
Melt liquor	66.1	2052	427	2.26	36	39
% change from W.R. sugar to melt liquor		127.5	139.9		140.0	160.0
% load in melt liquor from recycle		56.0	56.2		58.3	61.5

Average of six sets of samples.

Complete data sets for both refineries A and B are in Appendix A of the S.I.T., 1986 paper.

The recycling of non-sugars other than color is not considered here, although recycling of polysaccharides was pointed out in a 1978 study, and recycling of dextrans was calculated by Fowler in 1981, and is considered in another paper at this Conference (Clarke et al., 1986).

Melt sweet water in refinery A was made up of wash waters from filter muds, clarifier muds, and dust collectors; in refinery B, of wash waters from filter presses and dust collectors. The colorants in these are soluble color that was removed in the first pass through clarification and filtration. The effect of heat and time on the nature of these colorants, and the chemical changes that have taken place--similar to those affecting colorant in remelt--are expected to make the recycled colorant more difficult to remove on a second pass, but no experimental work has been conducted to verify this assumption.

The question of sources of colorant in melt liquor--how much is derived from sweet water and how much is developed by heat in the melter--has been the subject of recent experiments, reported herein. The heating process can cause polymerization of phenolic with other phenolic and/or amine colorant to create more intransigent color bodies, and can develop color precursors in both sugar and recycled material into visibly colored molecules. The very high response to tests for both phenolics and amino nitrogen in the melt sweet waters of both refineries emphasizes the labile

nature of their non-sugars.

In an attempt to quantify the effect of heat, a simulated or synthetic melt liquor was prepared from refinery samples of washed raw sugar and melter sweet water, without application of heat, and color and concentrations of phenolics and amino nitrogen in this simulated melt were compared to those of refinery melt.

Sampling difficulties--always present, as mentioned above--were particularly obvious in this comparison: for example, several sets of samples showed higher color in the synthetic liquor than in the heated melt. One sampling problem, pointed out by Tom Pearson of Imperial Sugar, occurs in the taking of a washed raw sugar sample: the sugar is usually taken from the top of the affination centrifugal, and so is washed rather less than the average sugar emerging from the centrifugal.

Very High Molecular Weight Color

An investigation into the presence of very high molecular weight color in raw sugar has yielded initial results on distribution in several raw and washed raw sugars.

The ICUMSA color measurement includes a filtration step where the sample solution, adjusted to pH 7, is filtered through a membrane filter of 0.45 μ . In this study, these filtrates are then ultra-filtered under pressure through membrane of molecular weight cut-off pore size equivalent to 20,000 daltons. The difference between color in this filtrate and the ICUMSA color will give an indication of very high molecular weight color in the sample. Only raw cane sugars have been examined at this point: indications are that cane sugar colorants have a much higher molecular weight range than beet sugar colorants (Shore et al., 1984). The high molecular weight colorants are suspected to be selectively occluded in crystal formation, and so are of especial importance in the final product. This study also is continuing.

Correlation of Color Measurements at pH 7 and pH 8.5

In the past few years, many samples of whole and washed raw sugars have entered the S.P.R.I. laboratories in connection with various studies. Color measurements have been made on many of these at both pH 7 and pH 8.5. Some washed raws have come from refineries; some raw sugars have been washed in the laboratory (Meade and Chen, 1985) and are probably overwashed when compared to an affined sugar. Correlations between the various measurements are reported here.

METHODS AND MATERIALS

Recycling of Colorant

Samples were obtained as 4-hour or 8-hour composites from refineries' regular sampling schedules, and stored under refrigeration until collected (on the same day as sampled) by S.P.R.I. Samples were then stored at 4° C until analysis. Sets of samples were obtained at various times over a period of several months, and analyzed as soon as possible.

Procedures for analysis of phenolics, amino nitrogen compounds, and color readings at various pH adjustments and wavelengths are as described in earlier papers from S.P.R.I. These tests were adapted from those devised by Dr. Peter Smith and Nancy Paton at CSR Ltd. (Smith and Gregory, 1971).

Simulated melt liquor was prepared by adding washed raw sugar to melt sweet water, with stirring without heat, until the Brix of refinery melt liquor was reached. The quantity of washed raw may be estimated by the equation:

$$20g \times Bx_{S.W.} + 100(Q) = Bx_{m.l.} (20 + Q)$$

where 20g is the quantity of sweetwater used,

$Bx_{S.W.}$ and $Bx_{m.l.}$ are Brix of sweet water and melt liquor,

respectively, and Q is weight of washed raw sugar.

Ultrafiltration apparatus was an MFS stirred cell, model VHP 25, 5 kg/cm² pressure with 20,000 M.W. cut-off dry type VH membrane (Micro Filtration Systems, Dublin, California).

Whole and washed raw sugar samples were obtained from refineries. Lab washed raw sugar samples were prepared using the Hawaiian method (Meade and Chen, 1985).

Color at pH 7 was read using the ICUMSA method (Schneider, 1977). Color at pH 8.5 was read on samples, prepared using the ICUMSA method, including membrane filtration, with pH adjustment to 8.5 ± 0.2 instead of 7.0 ± 0.2.

RESULTS AND DISCUSSION

Melter Studies

Results comparing color increase in refinery melt liquor samples to color increase in simulated melt liquor, made without heat, are presented in Tables 4A,B,C, and D for samples from a phosphatation-bone char refinery, where no remelt is added back to the melter. Data show that the increase in color from washed raw sugar to melt liquor is as anticipated from the earlier survey: the summary of data in Table 5 indicate an average increase of 57%. The source of this color is not clear from these results: certainly some of it develops from the high temperature in the melter: results indicate that from 41% to all, or 100% of the increase, can be ascribed to color formed by heating. Because known amounts of color are added in dilution water the figures indicating heat as the total source are suspect. There is such a wide range of numbers, over so few samples, that the study must be continued before any definite trends can be observed.

Table 6 shows one set of data from Refinery B. This set indicates that all the color increase comes from remelt and melter water, and none from applied heat--again, an unlikely situation that calls for further sampling.

Table 4A.--Samples from a phosphatation-bone char refinery
Refinery A
Set 1

Sample	Brix	420, pH 7	I.V.	Phenolics ppm	Amino N ppm
Raw sugar		3185	2.05	61	45
Washed raw		749	2.79	22	30
Dilution water	17.4	3061	2.16	47	27
Melt liquor	67.0	1607	2.17	34	27
Synthetic melt liquor	67.0	1046	2.34	22	16
% change, W.R. - M.L.		115%		55%	-10%
% change, W.R. - Sim. M.L		40%		0	-47%
% color increase from heat		75%		55%	--
% color increase from recycle		40%		0	-ve

Table 4B

Refinery A
Set 2

Sample	Brix	Color		Phenolics ppm	Amino N ppm
		420, pH 7	I.V.		
Raw sugar		4079	2.97	65	48
Washed raw		911	5.61	11	15
Dilution water	17.9	3569	2.98	56	17
Melt liquor	66.7	1683	3.07	28	29
Synthetic melt liquor	66.7	1371	4.10	16	9
% change, W.R. - M.L.		85%		155%	93%
% change, W.R. - Sim. M.L.		50%		45%	-40%
% color increase from heat		35%		110%	
% color increase from recycle		50%		45%	-ve

Table 4C

Refinery A
Set 3

Sample	Brix	Color		Phenolics ppm	Amino N ppm
		420, pH 7	I.V.		
Raw sugar		2688	1.69	39	14
Washed raw		602	2.89	7	11
Dilution water	20.4	2232	2.37	36	6
Melt liquor	68.8	919	3.31	8	10
Synthetic melt liquor	68.8	755	2.68	10	11
% change, W.R. - M.L.		53%		12.5%	
% change, W.R. - Sim. M.L.		20%		43%	
% color from heat		23%			
% color from recycle		20%			

Table 4D

Refinery A
Set 4

Sample	Brix	Color 420, pH 7	I.V.	Phenolics ppm	Amino N ppm
Raw sugar		3560	3.0	58	55
Washed raw		1073	2.9	18	20
Dilution water	13.6	2489	2.3	54	27
Melt liquor	66.6	1329	2.9	24	22
Synthetic melt liquor	66.6	1055	2.1	23	23
% change, W.R. - M.L.		24%		33%	10%
% change, W.R. - Sim. M.L.		-1.6%		28%	15%
% color from heat		24%			
% color from recycle		---			

Table 5.--Summary of data on color increase from recycled material
in melter at Refinery A

Set	1	2	3	4
% color increase from recycle	115	85	53	24
From heat	75	35	23	24
From melter water	40	50	20	--

Table 6.--Samples from a carbonatation/char refinery

Refinery B
Set 1

Sample	Brix	Color		Phenolics ppm	Amino N ppm
		420, pH 7	I.V.		
Raw sugar		5521	2.0	160	91
Washed raw sugar		1390	3.0	20	13
Remelt		8149	1.8	70	38
Hi Pur-S.W.	8.0	2209	2.0	91	70
Melt liquor	66.1	1878	2.3	23	18
Synthetic melt liquor	66.1	1892	2.5	24	15
% change, W.R. - M.L.		35%		15%	38%
% change, W.R. - Sim. M.L.		36%		20%	15%
% color from heat		--			
% color from recycle		36%			

Very High Molecular Weight Color

Table 7 outlines the distribution of very high molecular weight colorant in whole raw, affined and laboratory washed raw sugars. The lab-washing produces an over-washed crystal, with a higher proportion of in-crystal color than the refinery-affined crystal. Cane sugars are known to contain higher molecular weight colorant than beet sugars (Shore et al., 1984), and it is of interest to observe the relative amount of very high molecular weight material, since that goes into the crystal itself.

Table 7.--Ultrafiltration of sugar colorant

Sugar	ICUMSA (thru 0.45 u)	Color	
		Ultrafiltered (thru 20,000 m.w.)	% above 20,000 m.w.
A raw	3107	2013	35%
affined	534	388	27%
lab-washed	389	274	42%
B raw	3386	1469	56%
affined	932	593	36%
lab-washed	606	375	38%
C raw	3029	2380	21%
affined	506	365	28%
lab-washed	458	261	43%

The ICUMSA color test includes a filtration step through a 0.45 u membrane filter. Solutions thus filtered were then passed through an ultrafiltration membrane (tangential flow) with a cut-off at 20,000 m. wt., and their colors read. The difference in color readings is ascribed to color from molecules of weight greater than 20,000 daltons.

The levels thus measured may be expected to be rather high, because in each filtration as the membrane pores become blocked, molecules of weight less than the cut off point can be trapped on the filter. Nevertheless, the percents high molecular weight color are surprisingly high.

Some of this color may be expected in the molasses coating of the crystal, where air and heat have encouraged polymerization reactions. High molecular weight colorants are known to be preferentially occluded inside the crystal (Clarke et al., 1984; Shore et al., 1984), and the increase in % high m. wt. color from affined to laboratory-washed (with less molasses coating than the affined) emphasizes this observation.

This initial work gives positive indication for continuing the study. Some of the material trapped by the filter may be polysaccharide with smaller molecules of colorant either covalently bound or adsorbed. Certainly, dialyzed or alcohol-precipitated isolates of high molecular weight material from raw sugars always show a brown color. Roberts and Godshall in 1978, using dialysis, showed that from 37% to 49% of raw sugar colors and 37% to 78% of

the corresponding wash raw color were retained by membranes of 12,000 molecular weight. W. W. Binkley determined many characteristics of the high molecular weight colorant which he isolated from molasses (Binkley's browning polymer) (Binkley, 1970). The relatively new and rapid technique of tangential ultrafiltration should provide a tool for separation and further investigation into this important class of colorant.

Color Measurements at pH 7 and pH 8.5

A number of observations were made on raw and washed raw sugar colors, at pH 7 and at pH 8.5, using the solution at pH 7 adjusted to pH 8.5. It was of interest to see the correlation between the readings at the different pH's, when the sample solutions were similarly prepared. The sugars were from many different origins. Washed sugars were refinery affined.

Figure 1 shows the correlation between raw and affined colors at pH 7. As expected, the correlation coefficient is not particularly high at 0.62, though a statistical evaluation would no doubt reject at least two points as outliers. The purpose of this exercise was to compare the color readings on actual samples. The comparison between raw and washed raw colors at pH 8.5, shown in Figure 2, produced a similar correlation of 0.62, again with two probable outliers. These calculations, in both sets of which were used the same sugars, indicate that, with similar sample preparation, both pH 7 and 8.5 indicate equally the anticipated sugar color after affination.

Figure 3 shows the relationship between whole raw sugar color at pH 7 and that at 8.5, again where all samples were prepared with membrane filtration. The correlation coefficient of 0.96 indicates that the higher pH can provide a satisfactory color measurement for whole raw. That for washed raw, shown in Figure 4, of 0.91, is rather lower. Since these sugars came from a variety of sources, affination procedures, as practiced at different refineries, will have had a range of effects that lowers this correlation.

SUMMARY

This report presents an update on the study of colorant recycling in the refinery melt house, in which melt liquor is compared to simulated melt, prepared without heat, to determine the respective color contribution of melt sweet water and melter heating. Further testing is necessary.

Preliminary results on the very high molecular weight colorant as separated by tangential ultrafiltration indicate that the quantities of this relative to that of low molecular weight colorant increase inside the crystal.

420 nm pH7

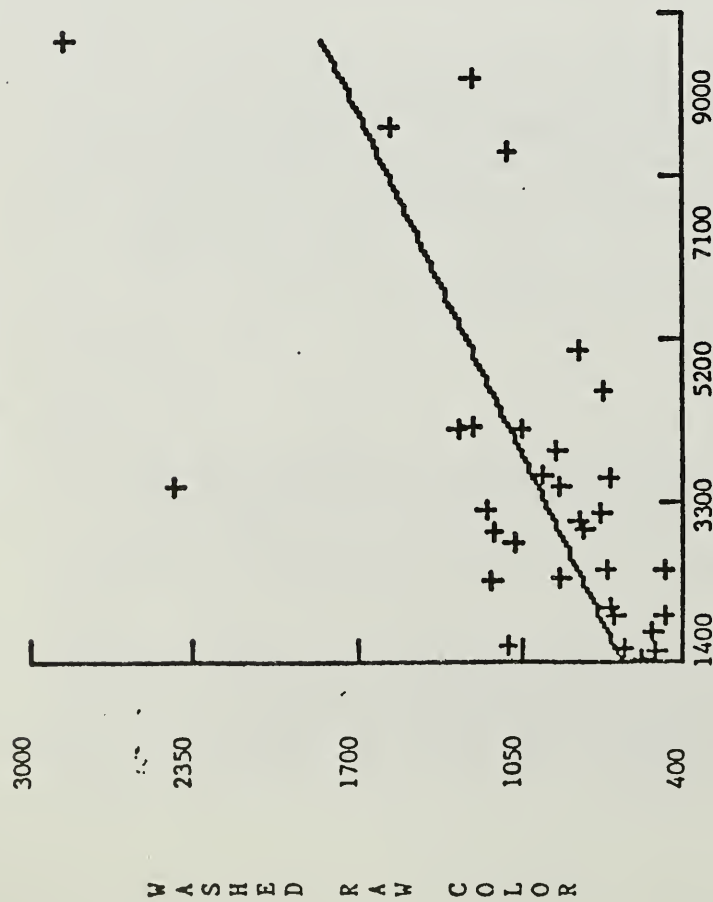


Figure 1.--Raw sugar color vs. washed raw sugar color at pH 7 (ICUMSA Method).

420nm pH8.5

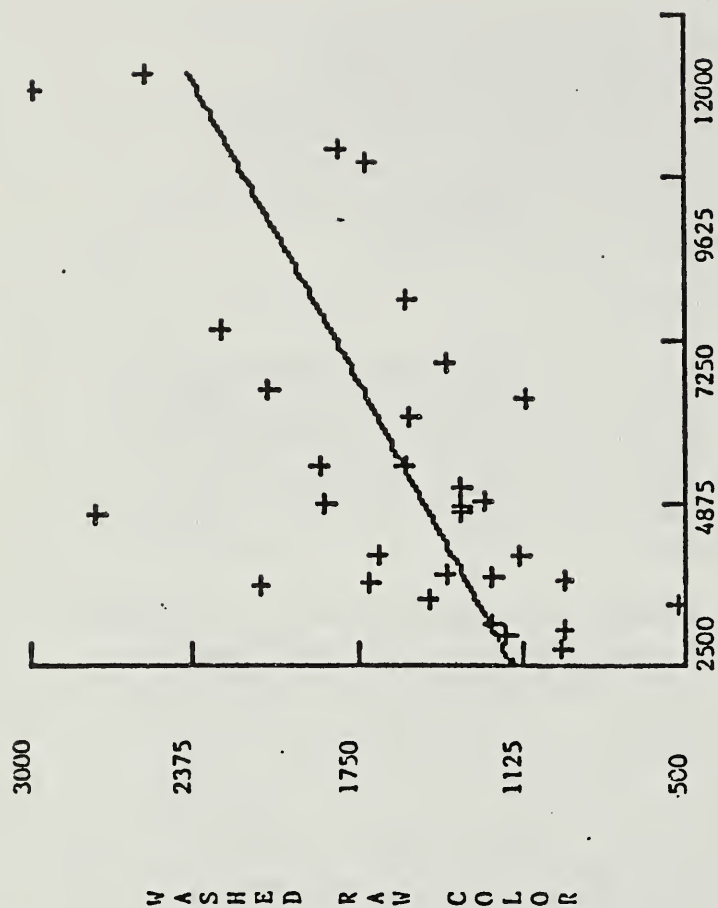


Figure 2.--Raw sugar color vs. washed raw sugar color at pH 8.5, after membrane filtration through 0.45 μ filter.

RAW COLOR pH7 vs 8.5

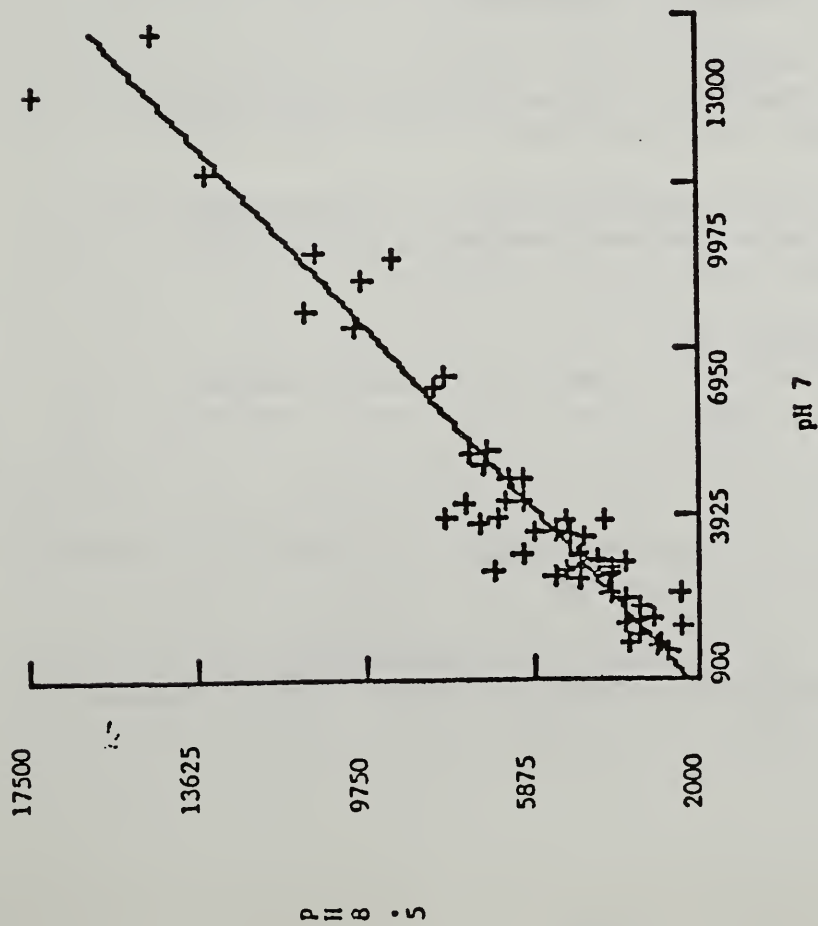


Figure 3.--Raw sugar color at pH 7 (ICUMSA Method) vs. color of same sugar at pH 8.5, both samples after membrane filtration through 0.45 μ .

WASHED RAW pH7 vs 8.5

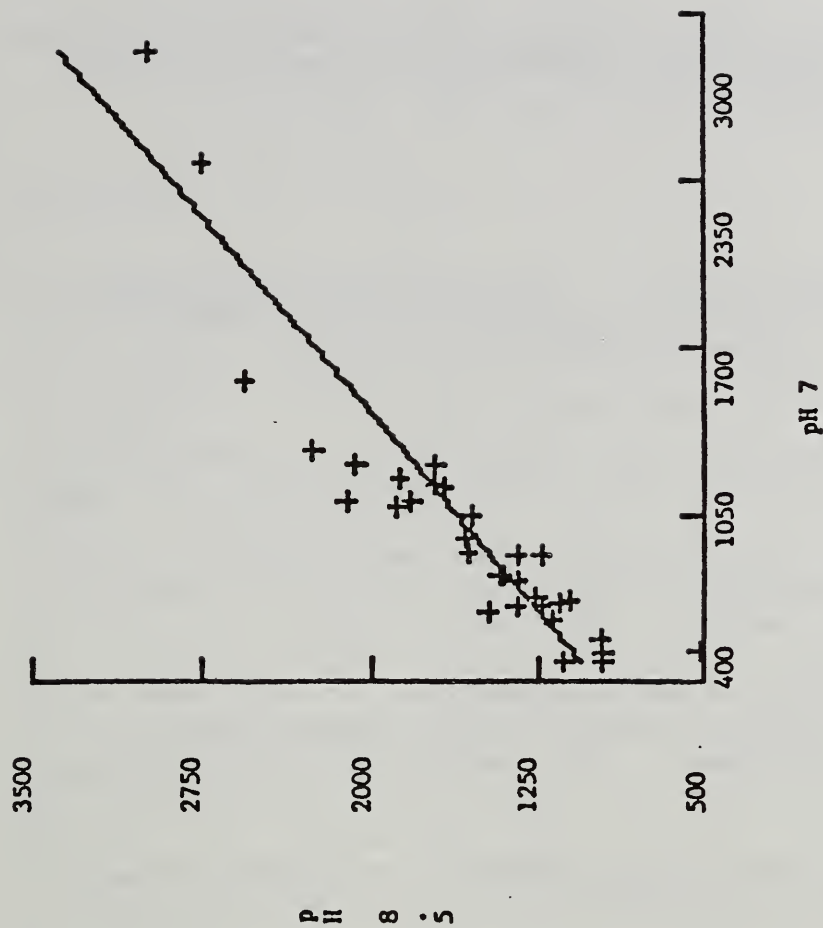


Figure 4.--Washed raw sugar color at pH 7 (ICUMSA Method) vs. color of same sugar at pH 8.5, both samples after membrane filtration through 0.45 μ .

Color measurements on a wide variety of sugars from many sources, both whole raw and affined, are compared at pH 7 and pH 8.5, on membrane-filtered samples.

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DISCUSSION

M. J. Daniels, British Charcoals - With regard to this color that you are recycling to the melter, and you hinted that it was a little simpler the first time around, would you care to comment on the possibility of removing or at least reducing the color from this source?

Clarke - Thank you. I didn't mention that. In our paper with Alan James, we had mentioned there, and I briefly review here, the possible benefit to the refinery of getting out some of this color before it's put back again. It has already been through once, and some of it has changed. Rather than put it in again, we thought it was a good possibility to consider a small clean-up process, for either sweet water or sweet water mixed with the rather nasty stuff that's probably better not put back into refinery process. We had proposed then, and I would now, for a small loading, to consider a small installation, using either a decolorizing agent that's used already in the refinery--any sort of resin system, carbon system, filtration, classic or deep bed, or a new system such as the type Dr. Frank was talking about this morning--something that would be appropriate to each specific case. We had also proposed that someone who, for example, used resins, might have spent resin that would otherwise be discarded that could be used for partial clean-up here. This sort of thing should be considered. No one is going to spend a terrific amount of money on it, I'm sure, but we wanted to put the thought into people's minds of what facilities they had at their disposal that could be used to reduce the over-all color load going into process.

A.P.G. Kieboom - About the chemical structure of the high molecular weight colorants, you said you were thinking about polysaccharides attached to small colorants. Are you also thinking that some high molecular weight molecules are already present in the beet or the cane, such as glycoproteins? Then, via Maillard reaction, these might give polysaccharide with small colorants attached to it.

Clarke - Yes, to both questions, particularly in beet sugar, for glycoproteins. There seems to be more evidence that this is a source of colorant in beet sugar, but, again, beet does not have the very high molecular weights that are found in cane.

In cane sugars, we know of two types of plant regulated color connected with polysaccharides at this time. We know that when polysaccharides are isolated, some small colorant molecules can be desorbed from them with an ethanol wash. This is material that has

been through dialysis and is above 12,000 molecular weight. That might be a simple chemical adsorption. There is some recent work by Geoff Richards, who had worked on cane polysaccharides in Australia and who is now in Montana on wood research, showing that there may actually be covalently linked polysaccharides and polyphenolics in some plant material. I think that is a possibility for us to investigate, too.

The other possibility that exists with all grass and cereal polysaccharides is phenolic groups attached as linking units. I think we may have some of these in cane sugars. Earl Roberts' indigenous sugarcane polysaccharide, for example, could have some esterified groups that may become colored in process and still remain attached to the polysaccharide.

NON-STARCH, SOLUBLE POLYSACCHARIDES OF SUGARCANE

Margaret A. Clarke, Earl J. Roberts and Mary An Godshall

Sugar Processing Research, Inc.

INTRODUCTION

The sugarcane plant contains many types of polysaccharides. Cellulose, pectin, and hemicellulose make up the cell wall material of the plant in stems and leaves. Starch and other glucans are part of the chain of food storage and synthesis in the plant. Other polysaccharides often found in the sugarcane result from microbial infections; for example, dextrans are products of Leuconostoc mesenteroides infection, and are not part of the plant system.

In 1972, Imrie and Tilbury published an excellent review of polysaccharides in, and associated with, sugarcane (Imrie and Tilbury, 1972). In subsequent years, several new polysaccharides which are native to the sugarcane plant have been identified. Some of these new compounds, for example, indigenous sugarcane polysaccharide (I.S.P.), which can be classified as a type of hemicellulose, are native to all cane plants (Roberts et al., 1964; Roberts et al., 1976; Blake et al., 1983; Blake and Clarke, 1984). Others, for example, the water soluble polysaccharide identified by Blake and Littlemore (1984; Blake and Littlemore (Part II)), are found only in stale cane or in cane that has been allowed to stand over from one crop to the next.

The reasons for interest in these polysaccharides are many: their effect on raw sugar production and quality parameters, such as pol, is the most familiar to processors. Their effect on refining efficiency and on the quality of refined sugars for uses such as soft drinks and alcoholic beverages is also well known. Knowledge of the structure of these compounds and their roles in biosynthesis and metabolism is important for the development of varietal characteristics such as high sucrose, lower starch and soluble polysaccharides, smaller quantity of fibre, and shorter time to maturity, among other factors.

Polysaccharides found in raw sugar come from either the cane plant, as, for example starch, or from products of invading microorganisms, as, for example dextrans. The total polysaccharides in raw sugar (Roberts, 1980) are frequently at much higher levels than the total of starches and dextrans. Historically, total soluble polysaccharides have been called "gums". Our recommended test for total polysaccharides is outlined in Appendix 1.

Table 1 shows analyses on several raw sugars, of various origins, and the proportions of unidentified soluble polysaccharide material in these sugars. The proportion of the latter is significant, ranging from 26% to 55%. It is of interest to processors, refiners, and cane growers to know what this material is, how it is produced by the sugarcane, and its effects on sugarcane processing and refining.

Table 1.--Polysaccharides in raw sugars

Sugar	Total polysacch., ppm	Dextran ppm	Starch ppm	Other soluble polysacch., %
A	1670	372	390	54.4
B	2030	775	141	54.8
C	2790	1584	382	29.5
D	1390	502	155	52.7
E	3120	1883	424	26.1

This paper will present a brief review on the soluble non-starch polysaccharides of sugarcane. Starches and dextrans will not be included, nor will the insoluble cellulose and hemicellulose materials, which make up the insoluble portion of bagasse.

Properties and structure of each type of polysaccharide are presented. The effects, or potential effects, of each type on processing are discussed.

Polysaccharides included in this review are: indigenous sugarcane polysaccharide (Roberts et al., 1964; Roberts et

al., 1976; Blake et al., 1983; Blake and Clarke, 1984), sarkaran and stand-over cane polysaccharide (Blake and Littlemore, 1984; Blake and Littlemore (Part II); Bruijn, 1973; Blake and Clarke, 1984; Blake and Clarke (Part IV)), Roberts' glucan (Roberts et al., 1985), beverage floc polysaccharides reported by Japanese workers (Miki et al., 1980; Miki, 1984), and some polysaccharides reported by Cuban workers (Cremata and Orozco, 1980; Cremata et al., 1983; Hormaza et al., 1983) and not identified as any of the above groups. As some of these compounds are not yet isolated or named, a temporary system of reference is used for them in this paper, e.g. Roberts' glucan; CP, galactomannan, from Japan.

Soluble Polysaccharides

Indigenous Sugarcane Polysaccharide (I.S.P.). In 1964, Roberts reported the presence of an arabinogalactan-type polysaccharide in fresh cane juice: this arabinogalactan often survives processing and appears in final products. Sutherland (Sutherland, 1959) in 1960 had found in cane syrups a hemicellulose-type polysaccharide, which appears to be a form of the arabinogalactan.

Roberts further elucidated the composition of the arabinogalactan (Roberts et al., 1976) and showed that it contained glucuronic acid units (Roberts and Godshall, 1978), which accounted for its negative charge at low pH's, as in acidified beverages. This polysaccharide became known as I.S.P. (indigenous sugarcane polysaccharide). It has a negative specific rotation (-46° to -50°), has a molecular weight ranging from 100,000 to 300,000 daltons, has a glucuronic acid content of 7 to 8% in the freshly extracted state, which decreases as a result of processing, and is extremely soluble. I.S.P. is one of the factors in a common type of acid beverage floc (Clarke et al., 1978).

The structure was further elucidated by Blake (Blake et al., 1983; Blake and Clarke, 1984), who compared I.S.P. from Louisiana cane to a similar polysaccharide from Queensland cane and identified I.S.P. as an arabinogalactan, with a backbone of beta -1,3 linked galactose residues, with arabinose and some galactose and other residues as side chains at the 6 position. The Australian arabinogalactan appeared to have a lower level of glucuronic acid (also substituted at the 6 position of galactose residues) than did the I.S.P.

All higher plants contain hemicelluloses as part of the cell wall structure of their non-endospermic parts (Wilkie, 1985). Sugarcane, a giant grass, is like other grasses in that most of its hemicellulose consists of polymers of xylans with other

minor sugar residues, and is insoluble and together with cellulose and lignins form bagasse. The I.S.P., or arabinogalactan, group may be regarded as a soluble hemicellulose and in the plant may serve to link phenolic and flavonoid residues to the insoluble cell wall glycans.

Another component of cell wall material common to plants is pectin which contains galacturonic acid residues in its polymeric structure. There are references in the old literature to "pectic substances" in sugarcane. Until recently (Roberts and Godshall, 1978), it was difficult to distinguish glucuronic from galacturonic acid when both were part of polysaccharide structures. The most common procedure was to titrate hydrolyzed polysaccharide for acid residues, with no identification of the type of acid. Recent studies at S.P.R.I. (Roberts et al., 1985) have shown no galacturonic acid in sugarcane polysaccharides, but only glucuronic acid. The old references to "pectic substances" probably result from confusion of this glucuronic with the more common galacturonic acid. Galacturonic acid or "pectic substance" is certainly found in sugarbeets.

I.S.P. is the non-sugar of greatest concentration in cane juice, after organic and amino acids (Roberts et al., 1985). I.S.P. and similar compounds can have a negative effect on polarization if they are not removed by lead acetate clarification. Initial studies at S.P.R.I. indicate that there is partial removal of these polysaccharides by lead acetate.

Sarkaran

In 1966, Bruijn, in an examination of the polysaccharides in cane allowed to become stale after harvesting, observed a glucose polymer in addition to dextran. This glucan, which he later named sarkaran, increased in concentration with the cut-to-crush time. Bruijn showed that sarkaran, which is very soluble and has a specific rotation of $+160^\circ$, is a linear glucan with approximately 75% α -1,4 and 25% α -1,6 bonds arranged as a polymer of maltotetraose and maltotriose (Bruijn, 1966; Bruijn (Part II); Bruijn (Part III), 1969). It has some similarity to pullulans, but is not a pullulan. Recently, Blake and Littlemore (Blake and Clarke, 1984; Blake and Littlemore, 1984) examined the soluble polysaccharides in cane that had been allowed to stand over from one crop year to the next and isolated a glucan which appeared to be similar to sarkaran. Intensive structural analysis by proton and carbon n.m.r., methylation analysis, and enzymic analysis led Blake to conclude that the glucan in standover cane is indeed sarkaran. Blake found sarkaran in molasses from refractory syrups from several factories crushing standover cane, verifying its structure by proton n.m.r., periodate oxidation, and enzyme

analysis. He described the specific arrangement of maltotetraose and maltotriose in the molecule.

Sarkaran is a problem in processing because it increases viscosity of molasses and syrups, appears to lower crystallization rate, and causes frothing (Blake and Littlemore, 1984). Sarkaran, with a specific rotation of $+160^{\circ}$ to $+170^{\circ}$, can increase juice polarization, as it is apparently not removed by lead acetate clarification (Blake and Clarke (Part IV), 1984).

Blake found sarkaran to be present in up to 0.13% concentration in standover cane in poor condition. In speculating on the origin of sarkaran, which increases in concentration as cane is stored after harvest, he proposed that sarkaran is a product of a microorganism, perhaps a yeast, rather than a plant product, because of the long induction period for its appearance.

Blake (Blake and Clarke, 1984) also published an assay procedure, using pullulanase digestion and subsequent colorimetric reaction of the reducing sugars produced, for sarkaran. He found errors from interference by reducing sugars and starch fragments. There may also be error caused by transglycosylation activity of the enzyme.

Roberts' Glucan

Very recently, Roberts of the S.P.R.I. group reported the isolation of a small glucan from fresh cane (Roberts et al., 1985). This glucan is present with I.S.P. in isolation procedures, and had heretofore been thought to be a low molecular weight component of I.S.P. This glucan is found in all cane, not only in stale or standover cane. It is low in molecular weight ($< 50,000$ daltons), and has a specific rotation of $+120^{\circ}$. Structural investigation by methylation, periodate oxidation, enzyme analysis, and proton nmr (190 MHz) showed a backbone of α -1,4 linkages, with about 12% branching as α -1,6 linkages. The glucan appears similar in structure to amylopectin, but is extremely water soluble, while amylopectin is quite insoluble. The very high degree of branching explains the solubility. The glucan produces a red-purple colour reaction with iodine (similar to the colour produced by amylopectin); but under polarized light and magnification does not show the Maltese cross pattern characteristic of amylopectin.

This glucan is probably a type of storage polysaccharide in the food chain of the cane plant, and may be an intermediate in the biosynthesis of sucrose, or the product of an alternate

biosynthetic pathway. Roberts (Roberts and Clarke, 1985) proposes that it is a plant glycogen, or phytoglycogen. It is present in quite small amounts (< 0.01% on cane) but the ratio of quantities of glucan to I.S.P. varies with age and variety of cane. Studies are underway to explain this observation, with the aim of increasing knowledge of the maturing process of the plant.

This glucan does not increase viscosity of syrups (Roberts and Clarke, 1985) and has little effect on polarization, so it does not appear to have any deleterious effect on processing. It does not produce acid beverage floc, and is near the lower limit of the molecular weight range to produce alcohol floc in cordials.

Galactomannan from Japan (CP)

In studying raw sugars that, when refined, produced beverage floc in carbonated beverages, Miki et al. at the Japan Sugar Refiners' Association isolated polysaccharide material that showed positive floc-forming potential (Miki et al., 1980; Miki, 1984; Cremata and Orozco, 1980; Cremata et al., 1983; Hormaza et al., 1983; Sutherland, 1959; Roberts and Godshall, 1978; Clarke et al., 1978; Wilkie, 1985; Roberts et al., 1985; Bruijn, 1966; Bruijn (Part II) 1966; Bruijn (Part III), 1969; Miki et al., 1975). The polysaccharide material, named CP, contained galactose, glucose, and mannose in varying ratios. Subsequent work (Miki, 1984) on structural analysis of the purified polysaccharide from CP, by gel filtration, methylation, periodate oxidation, and enzymic analysis, showed that it is a galactomannan, with two possible structures: (a) the galactomannan has a main chain consisting of alpha-1,6 and alpha-1,2 linked D-mannopyranose residues; single alpha-D-galactopyranose residues and alpha-D-mannopyranose residue are attached in the ratio of 3.0:1.0 to 86% of the alpha-1,6 linked D-mannopyranose residues in the main chain through alpha-1,2 links, or (b) the main chain of the galactomannan consists of only alpha-1,6 linked D-alpha-1,6 mannopyranose residues, 86% of which are branched at O-2. D-mannopyranose alpha-1,2 residues are alpha-1,2 linked in side chains that are terminated by non-reducing alpha-D-mannopyranosyl and/or alpha-D-galactopyranosyl groups. In this possibility, most or all of the alpha-D-galactopyranose and some non-reducing alpha-D-mannopyranose residues are considered to be attached through O-2 to alpha-D-mannopyranose residues of the main chain (Miki, 1984).

No indication is given of the source of this polysaccharide, whether from the cane plant or an external source.

Polysaccharide from Cuban Molasses

In 1980, Cremata and Orozco (1980) isolated a previously unreported polysaccharide from molasses of high viscosity by separation with cetyl trimethylammonium bromide and gel filtration. Carbon nmr, GLC, and mass spectrometry show a backbone of 1,3-linked glucopyranose residues, branching at C-6 galactose with arabinofuranosyl terminal groups, all in alpha-linkage.

No indication is given of the source of this polysaccharide, but it may be another aspect of the hemicellulose complex with storage glucans.

This laboratory and Cuban research workers, in work on the influence of polysaccharides as crystal habit modifiers, have identified low molecular weight (< 10,000 daltons) polysaccharides as among responsible factors for crystal elongation. No definite indication of structure or origin of these large oligosaccharides is given.

SUMMARY

A brief review has been presented of non-starch soluble polysaccharides of sugarcane. Among the compounds discussed with regard to structure, origin, and role in processing are sarkaran, I.S.P. and other arabinogalactans, Roberts' glucan, a galactomannan, and a copolymer of glucose with arabinose and galactose.

APPENDIX 1. S.P.R.I. TEST FOR ESTIMATION OF TOTAL SOLUBLE POLYSACCHARIDES

This procedure determines total soluble polysaccharides: dextran, starch, mannans, I.S.P. (arabinogalactans), and any other soluble polysaccharides in sugar or cane juice. The soluble polysaccharides are those of special concern to the processor and refiner because they remain in solution throughout processing and are present in final products.

The isolation procedure is based upon the precipitation of the polysaccharides from a sugar solution by alcohol. The precipitated polysaccharides are filtered off and the filter is washed with 80% V/V alcohol until free of sugar. The polysaccharides are dissolved out of the filter by boiling in 1% V/V sulfuric acid. The polysaccharide solution thus obtained is adjusted to a definite volume, filtered, and the mg of polysaccharides per ml of solution is determined colorimetrically.

APPARATUS AND MATERIALS

Apparatus

Millipore filter - 300 ml millipore filter holder with fritted glass bottom. Millipore filter - Type LS Teflon filter paper, 47 mm in diameter and pore size of 5 microns. Filter aid - Celite analytical filter aid. Volumetric flask - Several volumetric flasks of 200 ml and 250 ml. Flasks - Several 250 ml Erlenmeyer flasks. Funnels - Several short stem funnels 70 mm in diameter. Filter paper - Whatman No. 42 ashless filter paper 12.5 cm diameter, and Whatman No. 1 or No. 2 coarse filter paper.

Solutions

1% V/V sulfuric acid - dissolve 5 ml of concentrated sulfuric in 495 ml of deionized water. 5% W/V phenol solution - place 5 g of phenol in a 100 ml flask and add deionized water to the mark, and shake until dissolved. 80% V/V alcohol - measure 400 ml of absolute ethanol in a 500 ml flask, add 100 ml of water and stir. Alcohol - absolute ethanol. Concentrated sulfuric acid.

Preparation of sample solution

Dissolve 100 g of the sugar to be analyzed in 150 ml of deionized water and adjust the volume to 250 ml in a volumetric flask and let stand for 30 minutes. Filter about 40 ml of solution through coarse filter paper in a 70 mm funnel. For cane juice: filter 40 ml through coarse filter paper in a 70 mm funnel.

Precipitation of polysaccharides

Withdraw 10 ml of the solution with a pipet and place in a 100 ml beaker, add 0.5 g of celite analytical filter aid, stir, and add 40 ml of absolute ethanol. Filter the solution with suction on a Millipore filter using a type LS Teflon filter paper, 47 mm in diameter, and pore size of 5 microns. When the liquid has disappeared from the surface of the filter aid, wash the filter with 150 ml of 80% V/V alcohol to remove sugars. This is conveniently done with the 80% alcohol contained in a plastic wash bottle. The 80% alcohol should be carefully applied down the inside walls of the funnel in 20-25 ml portions, allowing each portion to disappear from the surface of the filter aid before adding the next.

Separation of polysaccharides

Quantitatively transfer the filter aid and filter paper to a 400 ml beaker and add 150 ml of 1% V/V sulfuric acid solution. Boil the mixture for 5 minutes. Remove the filter paper and rinse with water, allowing the rinse water to go into the beaker. Quantitatively transfer the contents of the beaker to a 200 ml flask, cool to room temperature, and dilute to the mark with deionized water. The volume of the filter aid is insignificant. Filter the solution through a Whatman No. 42 filter paper by gravity, discarding the first 10-15 ml of filtrate. The next 10-15 ml may be used for the determination. The soluble polysaccharides are in the filtrate. It is not necessary to filter the entire solution.

Development of color (phenol-sulfuric acid test)

Pipet a 2 ml aliquot of the polysaccharide solution into a 20 mm x 150 mm test tube and add 1 ml of a 5% aqueous solution of phenol. Ten (10) ml of concentrated sulfuric acid is then added at one time from a pipet with a large opening, preferably an automatic pipet. After the solution has cooled to room temperature (about 30 min.), the color is read on a spectrophotometer at 485 nm against a blank prepared in the same way as the sample except that 2 ml of water is used instead of the polysaccharide solution. The color determinations and blanks should be done in duplicate. If the percent transmission in the duplicates varies more than 2%, both should be repeated. The mg of glucose per ml of solution corresponding to the color reading is then determined from the standard curve. Take 90% of the glucose value to convert it to polysaccharide value.

Preparation of standard curve

Place 100 mg of pure glucose in a 1000 ml volumetric flask and make up to the mark with deionized water. For preparing the standard curve, dilute this stock solution as follows:

ml stock solution	dilute to	mg glucose/ml
10	100 ml	0.01
20	100 ml	0.02
30	100 ml	0.03
40	100 ml	0.04
50	100 ml	0.05
60	100 ml	0.06
70	100 ml	0.07
80	100 ml	0.08
90	100 ml	0.09
100	---	0.10

Place 2 ml of each solution in a 20 mm x 150 mm test tube, and add 1 ml of a 5% aqueous phenol solution to each tube. Then 10 ml of concentrated sulfuric acid is added all at once from a pipet with a large opening, preferably an automatic pipet. When the solutions have cooled to room temperature, the color is read on a spectrophotometer at 485 nm against a blank prepared in the same manner except that 2 ml of water is used instead of the glucose solution. The color readings are then plotted on graph paper. If the color is read as percent transmission, it must be plotted on semi-log paper of one cycle. If the color is read as optical density, the values are plotted on a square paper. The curve is used to determine the polysaccharides corresponding to the color measurement in an unknown solution.

INTERFERENCES AND SOURCES OF ERROR

The phenol-sulfuric acid reaction is extremely sensitive to all carbohydrate material including cellulose and starch. Every precaution must be taken to make sure that all glass apparatus is free from dust particles, pieces of tissue, etc., which will render the results erroneous. All glassware should be washed with deionized water immediately before use. The 5% phenol solution should be prepared fresh about every ten days. It is important that every detail of the procedure be followed, including use of the specified filter paper.

The teflon Millipore filters may be reused a number of times until they become plugged or develop a hole.

In this method the polysaccharides are precipitated by making the solution 80% V/V with alcohol. This precipitates almost all of the polysaccharides which are filtered off and washed free of sugar; the precipitated polysaccharides are partially hydrolyzed before the color forming reagents are added. In this way the high molecular weight polysaccharides are included. The phenol-sulfuric acid color reaction has been well established as a reliable method for carbohydrates.

The method is simple to perform and is rapid. It should be well suited for factory control work.

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GEL-LIKE MATERIAL PRODUCED IN THE PROCESSING OF COLOURED LIQUORS

Richard A. Kitchen
B.C. Sugar

Margaret A. Clarke
Sugar Processing Research, Inc.

A.J. DeLucca II
Southern Regional Research Centre

ABSTRACT

A white, gel-like material, made up of a water-soluble polysaccharide and a water-insoluble material, was isolated from coloured liquor process streams in a sugar refinery.

The water-soluble component was shown to be dextran; Leuconostoc mesenteroides could not be shown to be the source of this material.

The properties, characteristics, and structure of the water-insoluble material are discussed. The source of this insoluble material is considered, and reasons for its occurrence are presented.

INTRODUCTION

From March, 1985, to February, 1986, B.C. Sugar had a processing problem. This problem was restricted to the coloured liquor stream, and was essentially centred around the coloured liquor clarification system.

In the clarification process, as shown in Figure 1, the coloured liquor is phosphatated and limed in the "blow-ups" at 78° C. The pH 7.8 liquor is then Sweetland press filtered in the 75 - 78° C temperature range, and the 50° Bx liquor stored in coloured liquor feed tanks at pH 7.3 and 70° C. The liquor is transported from the feed tanks through the coloured liquor header system and inlet connection pipes to the cisterns, where it is decolourized by the adsorbents bone char or bone char-cane sorb mixture. The temperature of the liquor at this stage is near 70° C.

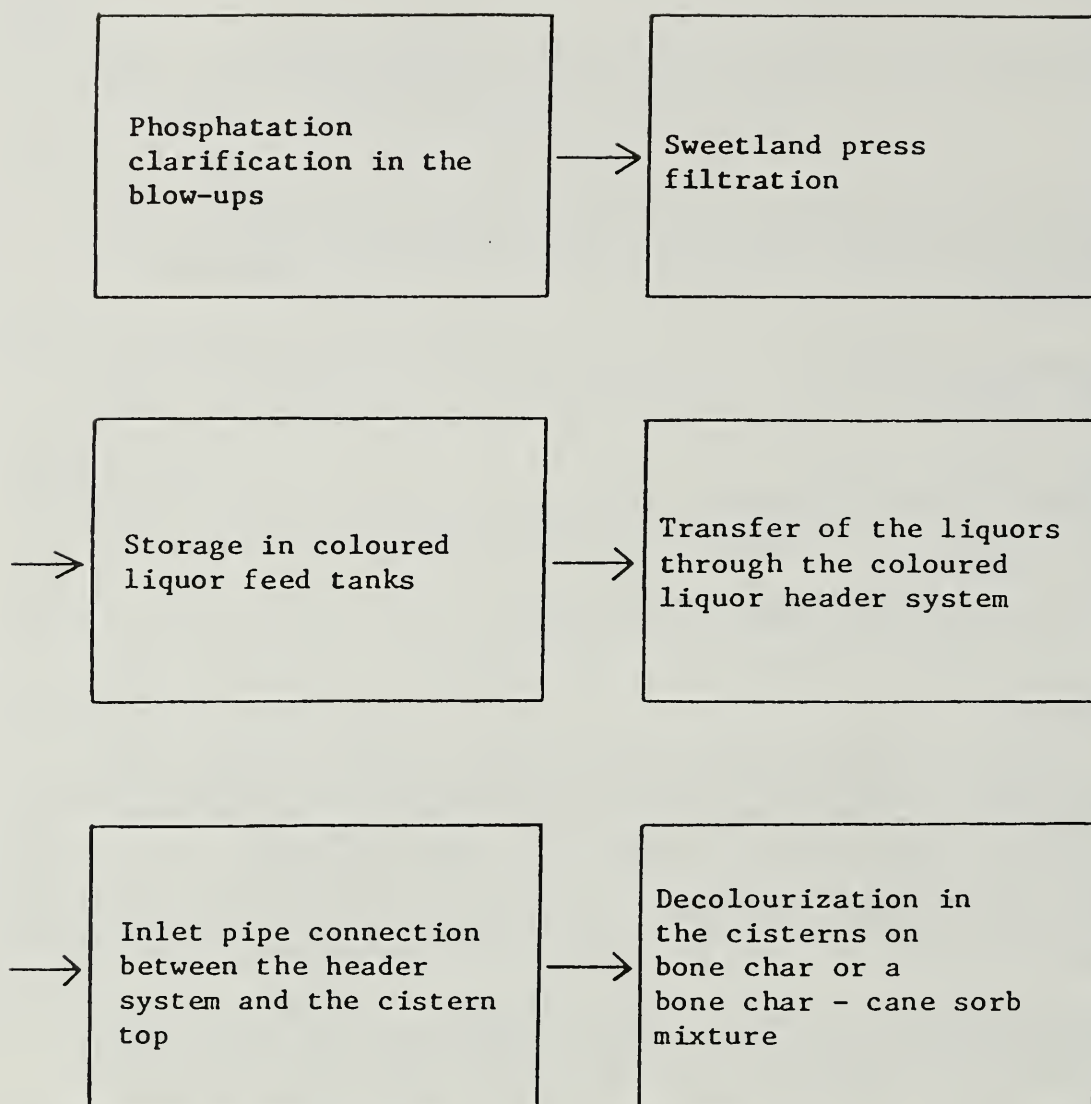


Figure 1.--Clarification steps in the coloured liquor stream.

It is the purpose of this paper to describe to you what caused the processing problems, where the problems occurred, their effects on processing, and how the problems were resolved.

The problems were caused by the formation of a white, gel-like material, which is shown mixed with coloured liquor and adsorbent in Figure 2.

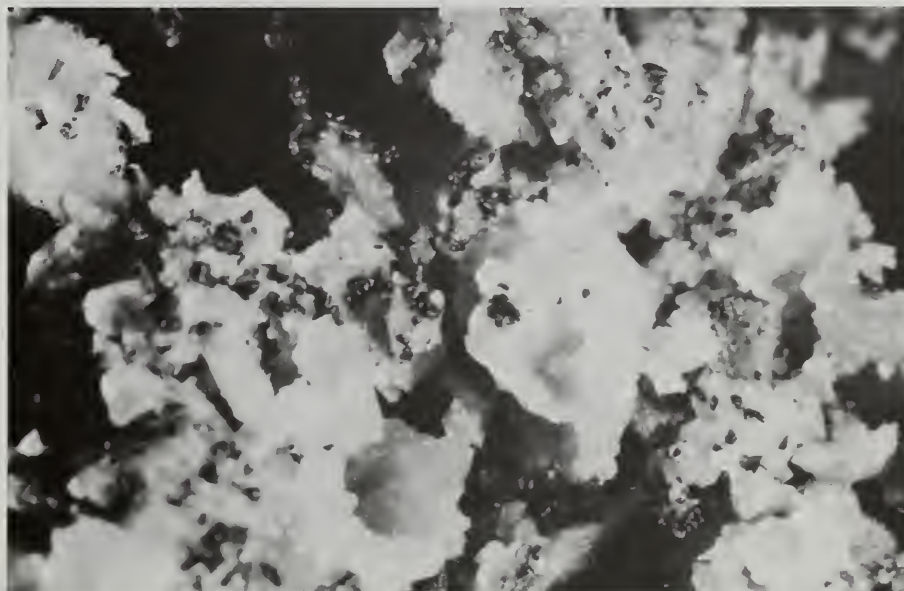


Figure 2.--White, gel-like material mixed with coloured liquor and adsorbent (sample obtained from top of adsorbent in cistern).

This gel-like material reached a depth of 4 - 6 inches in the coloured liquor feed tanks, was detected in the header system after dismantling, and was found on top of the adsorbents in the cisterns in depths of up to one foot. These various plant areas are shown in Figures 3, 4, 5, and 6.



Figure 3.--Coloured liquor feed tanks.



Figure 4.--Coloured liquor header system.

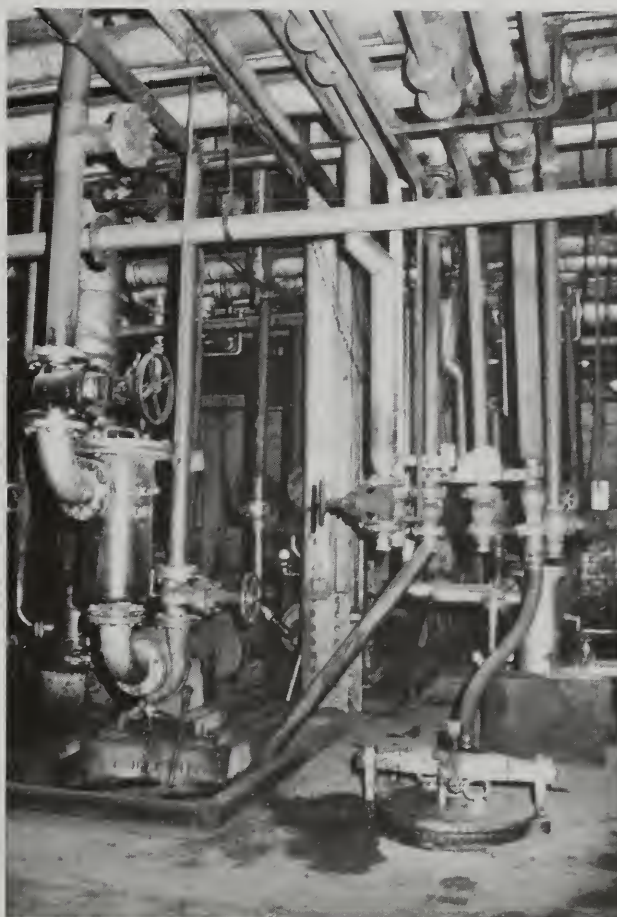


Figure 5.--Coloured liquor header and inlet connection pipe to cistern.

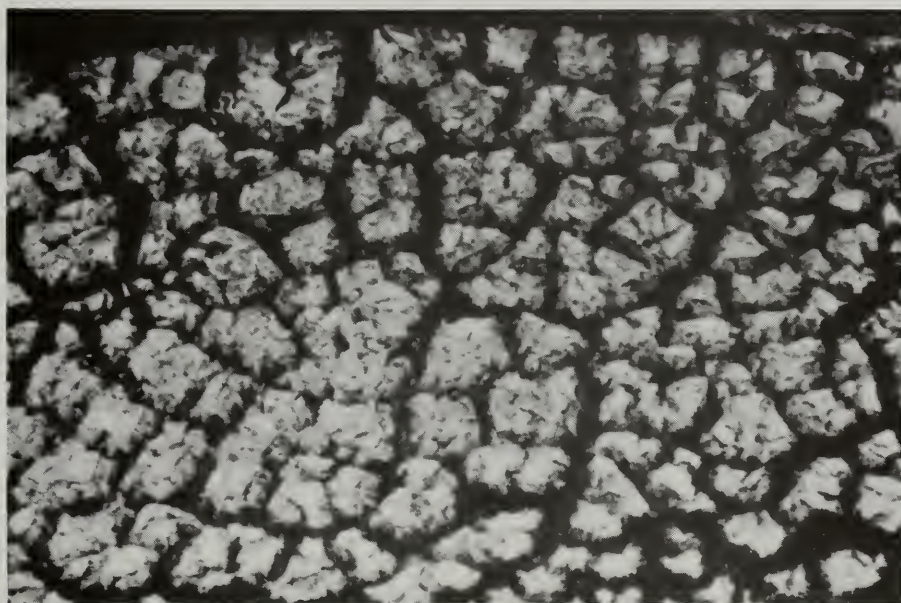


Figure 6.--White, gel-like material on top of the adsorbent in the cistern.

The effects upon processing were mainly centred around the bone char and bone char-cane sorb systems. Once the coloured liquor decolourization cycles had been completed, the adsorbent beds were washed with 90° C water, followed by 35 - 40° C water, and finally by 90° C water. Under normal circumstances, high pressure air was then passed through the beds to cause preliminary drying of the adsorbents. With gel on the adsorbent surface, however, very little air passed through, and very little drying occurred. Extra manual labour, therefore, was required to remove the adsorbents from the cisterns and, in particular, to pass the adsorbents through the driers and into the retort pipes in the kilns. The retort pipes, being incompletely filled, developed "hot spots," and these poor revivification conditions caused the colour and ash removal efficiencies of the adsorbents to drop.

The solution to these problems was to thoroughly clean out the feed tanks, header system, and inlet connection pipes with hot water and occasionally steam, to treat the entire system with solutions of sodium metabisulphite, and finally and most effectively to stop the storage of coloured liquors on the adsorbent beds over non-working week-ends. A later improvement was the replacement of the entire header system with a simplified, easier-to-clean version.

EXPERIMENTAL (CHEMICAL) - METHODS AND MATERIALS

Isolation of the whole gel. Gel samples were found to be insoluble in water, but soluble in 3 - 5% sodium hydroxide solutions. In samples containing no coloured liquor but small amounts of adsorbent, the impure gel was treated with water and magnetically stirred till the sample was fully suspended. The stirrer was then stopped, and the gel-like material at the top was removed by suction and set aside. This process was repeated until no further gel-like material could be separated from the denser adsorbent particles. The gel-containing fraction was then concentrated by evaporation and appeared as shown in Figure 7.



Figure 7.--Purified whole gel.

Standard plate counts on the whole gel. Inoculated nutrient agar plates and mycological agar (low pH) plates were incubated at 30° C to detect mesophilic bacteria and yeast - molds, respectively.

Total polysaccharide test and dextran test on the whole gel. The whole gel was dissolved in 3% sodium hydroxide solution, the solution neutralized with dilute hydrochloric acid, and then filtered through Whatman No. 1 filter paper. Three equal volume portions were removed from the filtrate; one portion was analyzed by the total polysaccharide test (Roberts, 1980), the second was analyzed by the dextran test (Roberts, 1983), and the third was evaporated to dryness to determine the percentage solids.

Acid hydrolysis of the whole gel. Gel samples were hydrolyzed in 2N sulphuric acid for 4 hours (reflux temperature) or 26 hours (90° C). The hydrolyzates were made slightly basic with barium hydroxide, treated with carbon dioxide gas until neutral, centrifuged, and the clean supernatants removed and analyzed by paper chromatography, GC and HPLC.

Paper chromatography of the whole gel hydrolyzates. The hydrolyzates were spotted on Whatman No. 3 chromatography paper, and irrigated in a descending fashion with ethyl acetate-pyridine-

water (8:2:1) for 48 hours, or with isopropanol-benzene-n-butanol (70:20:10)-water (20:3) for 28 hours. The dried papergrams were developed with alkaline silver nitrate reagent (Trevelyan, Procter, and Harrison, 1950).

GC analysis of the whole gel hydrolyzate. GC analysis of the trimethylsilyl ethers of the acid hydrolyzed whole gel was carried out on a Hewlett-Packard chromatograph model 5880 with a fused silica capillary column 12 metres in length coated with OV-101. The operating temperature was 175°C for 4 minutes, then programmed at 4°C/minute (Roberts et al., 1985).

HPLC analysis of the whole gel hydrolyzate. High performance liquid chromatography of the acid hydrolyzed whole gel was performed with a Waters 510 solvent delivery system, a Waters 410 Differential Refractometer detector, and a Waters 740 Data Module for recording and integration. The monosaccharide column (Bio-Rad) was an AMINEX HPX-87C (300 x 7.8 mm), operated at 85°C with water as solvent.

Percentage moisture on the whole gel. The whole gel was dried over sodium hydroxide pellets in an evacuated desiccator or dried in a convection oven at 60°C until a constant weight was obtained.

IR analysis of the dried whole gel. The infra-red analysis of the dried whole gel was carried out on a Perkin-Elmer Model 1130 spectrophotometer.

Specific rotation on the dried whole gel. The specific rotation was determined on a Hilger-Watts Standard Polarimeter equipped with a sodium light source. The solvent for the rotation was 5% sodium hydroxide solution.

X-ray powder diffraction on the dried whole gel. The X-ray powder diffraction was performed on a General Electric Model XRD-5 Diffractometer.

Total polysaccharide test and dextran test on the dried whole gel. The dried whole gel was dissolved in 3% sodium hydroxide solution, the solution neutralized with dilute hydrochloric acid, and then filtered through Whatman No. 1 filter paper. Portions of the filtrate were analyzed by the total polysaccharide test (Roberts, 1980) and by the dextran test (Roberts, 1983).

Separation of the whole gel into components. A sample of whole gel contaminated by coloured liquor, as is shown in Figure 8, was separated into one water soluble and two water insoluble components, as is shown in Figure 9.

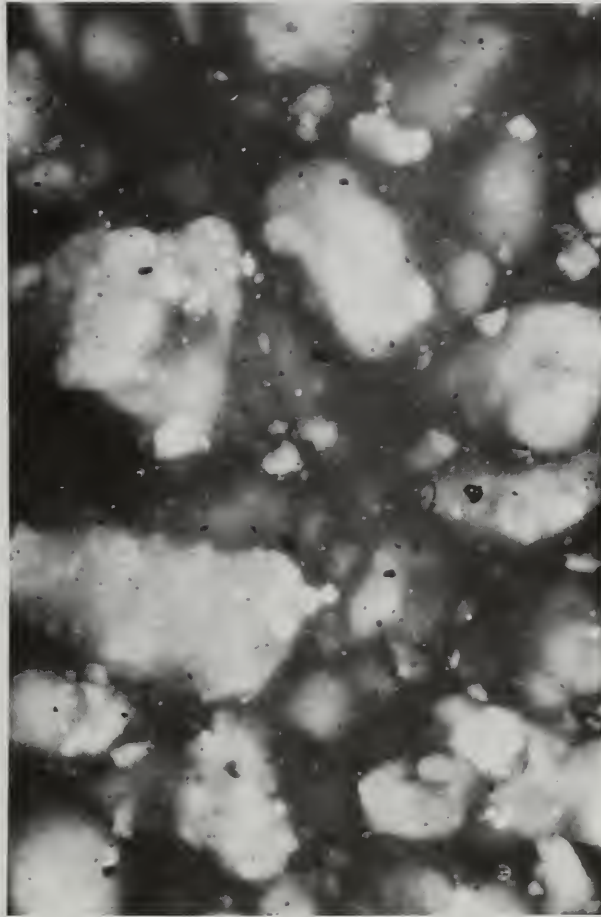


Figure 8.--Gel sample contaminated
by coloured liquor (sludge in
feed tank).

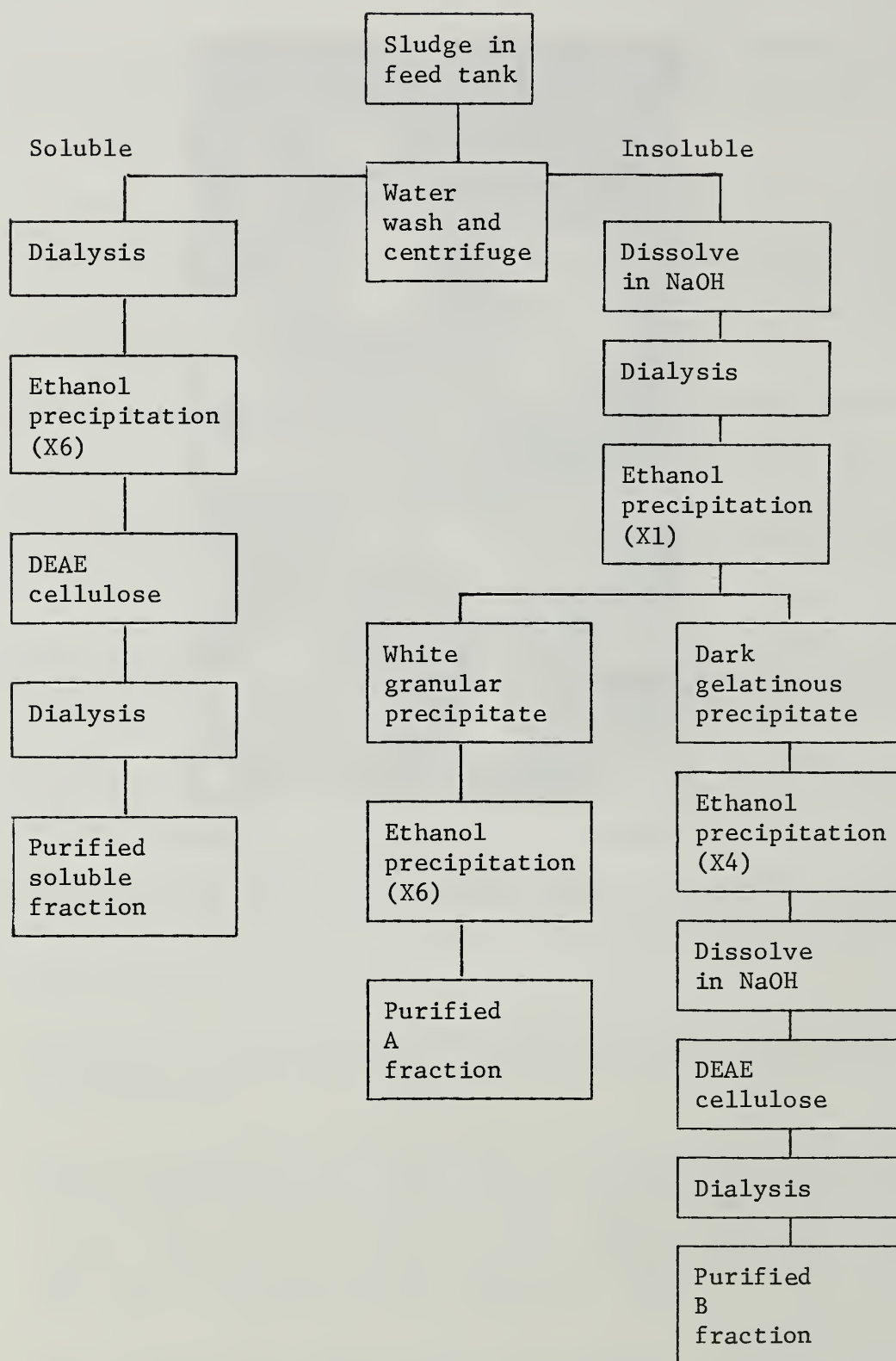


Figure 9.--Separation of whole gel into soluble and A & B insoluble fractions.

The purified fraction A is shown in Figure 10.



Figure 10.--Purified fraction A, a white, granular material.

An impure fraction B is shown in Figure 11.



Figure 11.--Impure fraction B, a heavy, rubber-like material.

The purified fraction B is shown in Figure 12.

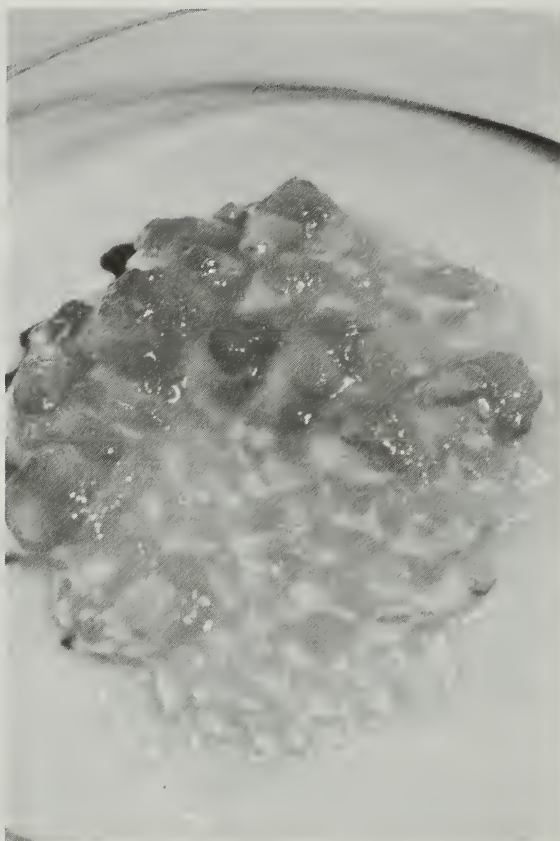


Figure 12.--Purified fraction B, a light orange coloured, rubbery material.

Dialysis on the separated components of the whole gel. The solutions were dialyzed in 3,500 or 12,000 - 14,000 molecular weight cut-off bags using flowing deionized, toluene-saturated water and a reciprocating table shaker for 100 hours.

Alcohol precipitations on the separated components of the whole gel. The aqueous solutions were made up to 65% ethanol by volume, and the precipitate isolated by centrifugation or by removal of the excess solvent. The precipitate was usually redissolved in water and the alcohol precipitation procedure repeated.

Decolourization of the separated components of the whole gel. The solutions were decolourized by gently stirring with an equilibrated, aqueous mixture of DEAE cellulose (DE 52; weight component - weight cellulose 1:1).

Acid hydrolysis of the separated components of the whole gel. The separated components were hydrolyzed by two different methods.

1. The solid or gel-like sample (200 - 500 mg) was treated with 2 ml of cold 72% sulphuric acid solution (Roberts et al., 1986), the mixture stirred at room temperature until complete solution occurred, then left standing for a total hydrolysis time of 1 hour. The solution was diluted with ice cold water to 10 ml, and neutralized with barium hydroxide and carbon dioxide gas, as described previously. Centrifugation gave a clear supernatant which was subjected to HPLC analysis.

2. The solid or gel-like sample (200 - 500 mg) was treated with trifluoroacetic acid (TFA, 0.3 M, 10 ml) and heated at 100° C for 30 minutes as described by Koizumi et al. (1985). The solution was then cooled to room temperature, diluted with 8 ml water, and treated with small portions of Amberlite IR-45 weakly basic anion exchange resin until the pH became neutral. The resin was removed by filtration with Whatman No. 42 filter paper, and the clear filtrate analyzed by HPLC.

HPLC analysis of the hydrolyzed components of the whole gel. High performance liquid chromatography of the hydrolyzed components of the whole gel was carried out on Waters equipment, as described previously. The monosaccharide column (Bio-Rad) was an AMINEX HPX-87C, operated at 85° C with water as solvent; the disaccharide column (Bio-Rad) was a Bio-Sil Amino 5S (250 x 4 mm), operated at 25° C with acetonitrile-water (82:18) as solvent.

Percentage moisture on the separated components of the whole gel. The separated components were dried in a convection oven at 40° C, and then in an evacuated desiccator over sodium hydroxide and phosphorous pentoxide until constant weights were obtained.

Specific rotation on the separated components of the whole gel. The Hilger-Watts Polarimeter, as described previously, was used; the solvent for the rotations was 2% sodium hydroxide solution.

EXPERIMENTAL (MICROBIOLOGICAL) - METHODS AND MATERIALS

Isolation of bacteria from a contaminated adsorbent sample. A 0.5 g sample of contaminated adsorbent was placed in 10 ml of sterile 50% yellow sugar broth and incubated at 50° C for 72 hours. After incubation, the cultures were streaked for isolation on triple sugar iron (TSI) agar plates, which also were incubated at 50° C. After 24 hours, the plates were examined, individual colonies showing acid production were selected, transferred onto TSI agar slants, and incubated at 50° C for 24 hours.

Identification of the isolated bacteria. The isolates were gram-stained and examined. The cultures were inoculated onto slants of phenyl red agar base amended with 1% carbohydrate. These slants were incubated at 50° C for 24 hours, and the reaction of the

isolates with the various carbohydrates was noted. A schematic of the microbiological procedures is shown in Figure 13.

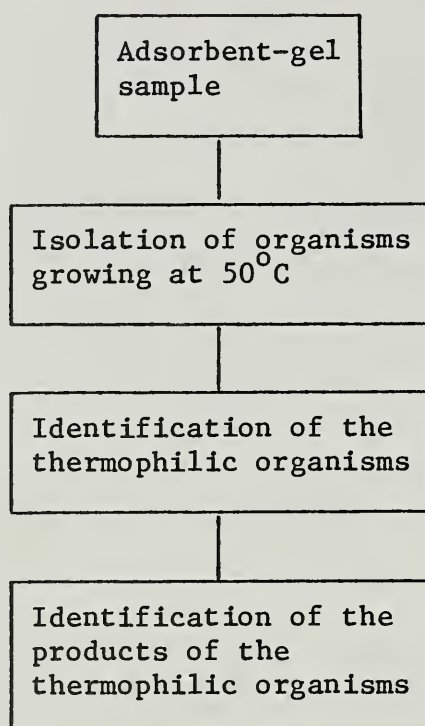


Figure 13.--Microbiological testing of an adsorbent-gel mixture.

Photography of the thermophilic bacteria. The isolates and a culture of Leuconostoc mesenteroides were photographed with a Zeiss Ultraphot camera-microscope apparatus, the magnification being 1000 X.

RESULTS (CHEMICAL) AND DISCUSSION

Repeated tests for water soluble dextran by the copper method (Roberts, 1983) were negative. Tests on later gel samples also indicated that if the gel was well washed on the adsorbent bed, no water soluble dextran was detected. Solubilization of these same

samples with 3 - 5% sodium hydroxide solution, followed by neutralization with dilute hydrochloric acid, did give soluble products and positive dextran tests. Samples which had not been on the adsorbent bed, e.g. feed tank sludge and header system samples, were found to contain water soluble substances which gave positive dextran tests.

A combination of the plating procedures and dextran-total polysaccharide tests was used to locate the problem areas in the refinery. The results from the dextran and total polysaccharide tests are shown in Table 1.

Table 1.--Dextran and total polysaccharide tests on gel-containing samples from the plant.

Raw sugar or coloured liquor sample	Dextran (ppm)	Solvent	Total polysaccharide (ppm)	Solvent
February/85 raw sugar	250	H ₂ O	1,035	H ₂ O
September/85 raw sugar	195	H ₂ O	-	-
A-side cistern	0	H ₂ O	-	-
Ex Sweetland press	1,300	H ₂ O	8,900	H ₂ O
Sludge in feed tank	32,000 28,000	H ₂ O NaOH	32,000 28,000	H ₂ O NaOH
Header system	21,000 20,000	H ₂ O NaOH	- -	- -
A-side cistern	12,000	NaOH	11,000	NaOH
B-side cistern	0 3,500	H ₂ O NaOH	- -	- -

1-4 Refers to a specific raw sugar cargo and its processing.

5 Sodium hydroxide solutions (3-5%), followed by neutralization with dilute hydrochloric acid.

6 Plating indicated bacteria and yeasts too numerous to count, molds were fifty-two.

The results from the dextran-total polysaccharide tests and the regular plating experiments indicated that the areas in the plant where dextran was formed were after the Sweetland presses up to the adsorbent surfaces in the cisterns. The production of dextran obviously was caused by a microbiological infection, bacteria or yeasts, but could not be clearly attributed to the bacteria

Leuconstoc mesenteroides. The detection of dextran was important, but not as important as the properties of the gel and their effect on processing. These properties are recorded in Table 2.

Table 2.--General properties of the whole gel.

Properties	
Appearance	White, rubbery gel
Solubility	Insoluble water, soluble 3 - 5% sodium hydroxide solution
Water content	92.2%
Specific rotation	$\left[\alpha \right]_D^{20} + 181.4^\circ$ in 5% sodium hydroxide solution (c = 0.0024 - 0.0030 g/cc)
Crystallinity	Non-crystalline (X-ray powder diffraction)
Composition after hydrolysis	Glucose, mannose (70:30), isomaltose, and other oligosaccharides (paper chromatography, HPLC, GC)
Gelation	Colloidal suspension (2% w/v water) formed on a gel on heating to 80 - 85° C.
Microanalysis percent components	C 39.07 (38.01), H 6.34 (6.11), N 0.23 (< 0.05), O 48.97 (51.08), empirical formulas CH_2O
IR analysis	Absorptions for C-OH and C-H

The whole gel contained very little solid material, the major portion being composed of water. Thus, very small amounts of the polysaccharide produced very large quantities of the gel. It was the combined properties of water insolubility, heavy hydration, and gelation which caused most of the processing difficulties in the plant. The adsorbents, after washing, were given a preliminary drying by air. This procedure was severely affected by the presence of the polysaccharide gel, which in turn affected the final drying in the driers and the revivification in the kilns.

The gelation characteristics were somewhat similar to those shown by curdlan, a β -(1-3) linked glucan, which has been reported to produce firm, resilient gels on heating (Harada et al., 1968; Maeda et al., 1967). The whole gel was shown to be an α -linked polysaccharide; the detection of isomaltose after hydrolysis and the high positive specific rotation value (Jeanes et al., 1954) being indicative of such a linkage. The structure of the whole gel, however, has not yet been determined.

The separation of the whole gel into one soluble and two insoluble components involved separation and purification techniques such as water extraction, base solubilization, dialysis, ethanol precipitation, and DEAE cellulose decolourization treatments, as shown in Figure 9. The general properties of the three components are listed in Table 3.

Table 3.--General properties of the soluble and A and B insoluble components of the whole gel.

Properties	Soluble	A insoluble	B insoluble
Appearance	Brown, granular	White powder	Light orange gel
Solubility	Soluble in water and sodium hydroxide solution	Dissociates in boiling water, soluble in sodium hydroxide solution	Insoluble in water, soluble in sodium hydroxide solution
Water content	8.7%	9.8%	90.5%
Specific rotation $[\alpha]_D^{20}$ in 2% sodium hydroxide solution	+143.0 (c=0.0020 g/cc)	+172.5 (c=0.0108 g/cc)	+163.8 (c=0.0038 g/cc)
Composition (two different hydrolysis methods)	Glucose, mannose isomaltose, and other mono- and oligosaccharides (HPLC)	Glucose, mannose isomaltose, and other mono- and oligosaccharides (HPLC)	Glucose, mannose isomaltose, and other mono- and oligosaccharides (HPLC)

Table 3.--General properties of the soluble and A and B insoluble components of the whole gel --- Continued.

Properties	Soluble	A insoluble	B insoluble
Gelation	Slight thickening on heating to 80-85° C	No gelation on heating to 80-85° C	Thickening on heating to 80-85° C
Microanalysis			
C	42.13 (42.01)	40.18	40.96 (40.87)
H	6.54 (6.51)	6.35	6.54 (6.45)
N	0.56 (0.53)	0.07	0.18 (0.13)
O	49.64 (49.64)	48.40	51.27 (51.27)

Empirical formula for all three compounds CH_2O

After hydrolysis, HPLC showed that the soluble polysaccharide and the A and B insoluble polysaccharides contained the same carbohydrates, glucose in highest concentration, followed by small amounts of mannose. Other monosaccharides were also indicated, their concentration being equal to or greater than that of mannose. Isomaltose and other oligosaccharides were found in all three samples; unfortunately, the oligosaccharides could not be sufficiently resolved to be identified with certainty. In preliminary work, using a modified HPLC technique of Nikolov et al. (1985), standard α -linked oligosaccharide samples could be separated, and their retention times compared to cellobiose.

The solubilities of the insoluble polysaccharides were consistent with that of the whole gel, while the water content values showed that only the B components contained an unusually high degree of hydration. This component also thickened on heating, but did not

form a gel. The detection of isomaltose, after hydrolysis, and the high positive specific rotation values indicated that the three components were each α -linked, as found for the whole gel.

RESULTS (MICROBIOLOGICAL) AND DISCUSSION

Plating of samples of the first gel produced (March, 1985) did not show spherical, lens-shaped cells characteristic of Leuconostoc mesenteroides, but bacteria and yeasts counts were extremely high, while molds were low or non-existent.

The isolates, obtained by the procedures described in the Experimental section, were inoculated onto slants of phenyl red agar base amended with 1% carbohydrate and incubated at 50° C. The reaction of the isolates is recorded in Table 4.

Table 4.--Biochemical characteristics of the thermophilic isolates from contaminated adsorbent and standard Leuconostoc mesenteroides.

Substrates and characteristics	9	Isolates		7B	<u>Leuconostoc mesenteroides</u>
		11	12		
arabinose	-	-	-	-	+
cellobiose	+	+	+	+	+
fructose	+	+	+	+	+
glucose (acid)	+	+	+	+	+
glucose (gas)	-	-	-	-	+
lactose	-	-	-	-	+
maltose	+	+(slow)	+(slow)	+	+
mannitol	-	+	+	-	+
mannose	+	+	+	+	+
melizitose	-	-	-	-	
rhamnose	-	-	-	-	
ribose	-	+	+	-	+
salicin	+	-	-	+	+
sorbitol	-	-	-	-	
sucrose	+	+	+	+	+
trehalose	+	+	+	+	+
xylose	-	+	+	-	+
esculin	+	+(weak)	+(weak)	+	+
catalyase	+	+	+	+	-
growth at 48° C	+	+	+	+	-
cellular morphology	rod	rod	rod	rod	coccus
nitrate	-	-	-	-	-
growth at 25° C	-	-	-	-	+

The results showed that the four isolates were entirely different in their reactions and their cellular morphology and in their growth temperatures to standard Leuconostoc mesenteroides. The isolates 9 and 7B gave identical reactions, as did the isolates 11 and 12, while the properties of all four isolates clearly indicated they were bacillus. The 9 and 7B isolates were identified as Lactobacillus jensenii, while the other two thermophilic bacillus have not yet been identified. The difference in cell shapes between standard Leuconostoc mesenteroides and the thermophilic bacillus is shown in Figures 14, 15, 16, and 17.

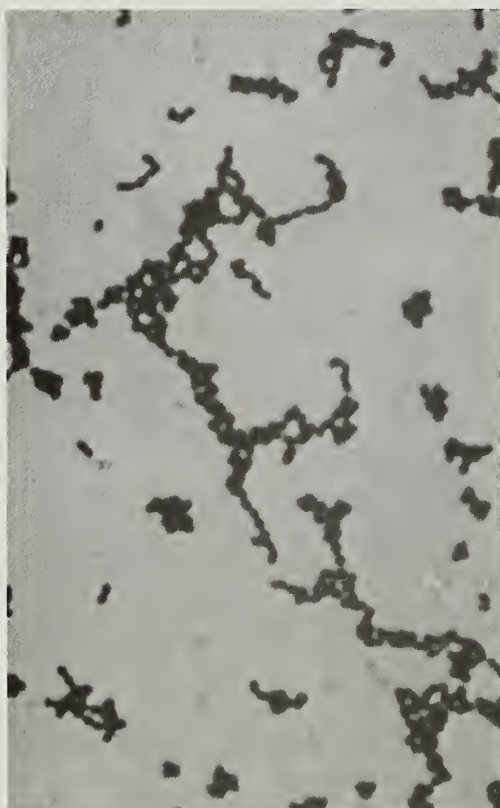


Figure 14.--Leuconostoc mesenteroides.

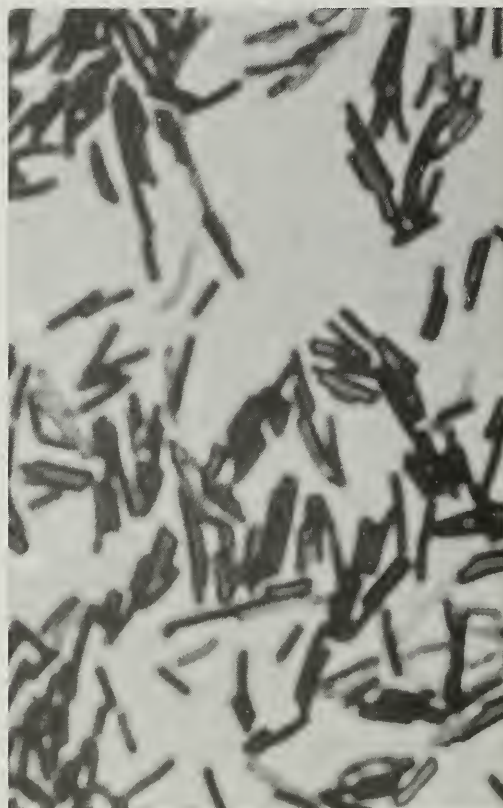


Figure 15.--Isolate 11, a thermophilic bacillus.

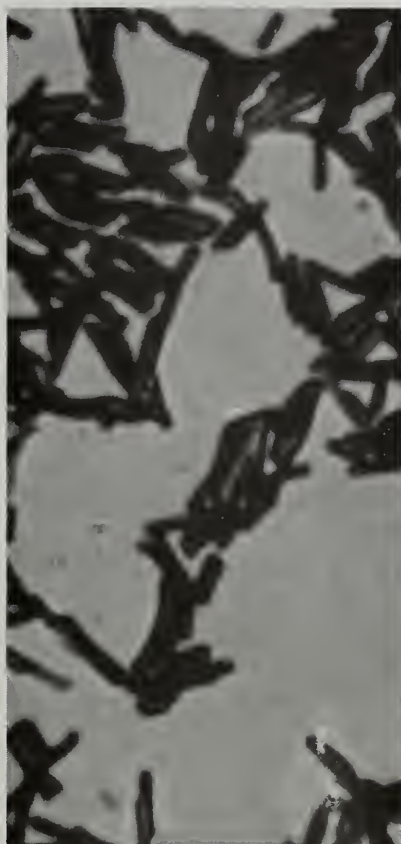


Figure 16.--Isolate 9,
Lactobacillus jensenii.

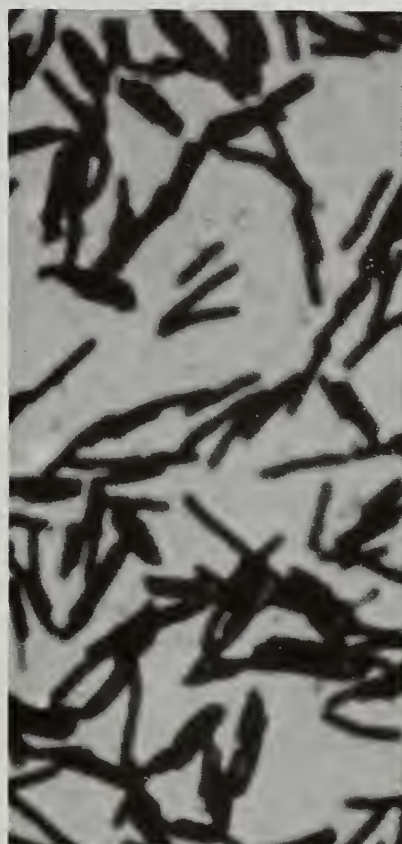


Figure 17.--Isolate 7B,
Lactobacillus jensenii.

The products formed by the four bacillus consisted of both water soluble and water insoluble components. Both of the components were polysaccharides; the insoluble polysaccharides from Lactobacillus jensenii were presumably dextrans, as they reacted with the alkaline copper reagent (Roberts, 1983).

SUMMARY AND CONCLUSIONS

The whole gel sample was not homogeneous, as it was composed of both soluble and insoluble polysaccharides.

It is proposed that the gel was produced by organisms belonging to the bacillus family, two being confirmed as Lactobacillus jensenii.

The gel itself was essentially polysaccharide in nature, the polysaccharides being composed of glucose as major component, mannose, and traces of other sugars.

Work is in progress on the further identification of the gel components.

ACKNOWLEDGMENTS

The speaker would like to thank the management of B.C. Sugar for their interest and their support in the preparation of this paper. Thanks are also due to Mrs. Anne Kitchen for her diligent laboratory work, to Dr. Dan O'Connell for his excellent slides and photographs, and to B.C. Sugar and S.P.R.I. personnel for their kind assistance.

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DISCUSSION

Richard Riffer, C and H Sugar - Dr. Kitchen, I realize that it's very difficult to do molecular weight determinations on insoluble material, but I wonder if you would comment on at what molecular weight you're likely to get an insoluble, gel-like material rather than a soluble one.

Kitchen - I think that the type of linkages in a polysaccharide are more important in determining the solubility than the molecular weight. Two types of polysaccharides which illustrate this point are the dextrans, whose solubility increases with an increasing proportion of α -1,6 linkages (Sidebotham 1974), and the curdlans, whose solubility decreases with an increasing proportion of α -1,3 linkages (Harada et al. 1968). Dextrans, as well, decrease in solubility with an increasing proportion of α -1,3 linkages (Sidebotham 1974). Eventually we will determine the molecular weight of our gel-like material, but our first priority is to determine the type and the percentage content of each linkage in that material.

Nicholas W. Broughton, British Sugar plc - When you were seeking to identify the organism, you did incubations at only one temperature, 50° C. Could you tell us how this temperature relates to the temperature of various process operations, and also to the optimum temperature for growth of Leuconostoc mesenteroides?

Kitchen - The problem occurred in a specific area of the refinery, from the colored liquor feed tanks through to the adsorbent cisterns. This section of the plant contained liquor at 70° C, certainly no lower than 65° C. Our feeling was that we were dealing with a thermophilic bacteria and a fermentative breakdown. We therefore picked the higher incubation temperature for our cultures to more closely simulate the refinery conditions.

Leuconostoc mesenteroides grows in the 10-37° C range, with optimum growth occurring near 20-25° C. Generally the bacteria do not withstand heating to higher temperatures, but in sugar factories the slimy cultures can encapsulate the bacteria and they can survive heating to 80-85° C (Bergey 1974). We did detect soluble dextrans in the feed tanks and header system, but none on the decolorizing adsorbent beds, presumably because they were washed out of the insoluble, gel-like material.

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THE USE OF REVERSED-PHASE CHROMATOGRAPHY IN CARBOHYDRATE ANALYSIS

Eero Rajakyla

Finnish Sugar Co., Ltd.

INTRODUCTION

The analysis of carbohydrate syrups obtained by different processes is normally carried out by high performance liquid chromatography (HPLC), which is rapid and convenient. Column packings used include polystyrene-based cation (Scobell et al, 1977; Ladisch et al, 1978; Fitt et al, 1981; Scobell and Brobst, 1981; Dunmire and Otto, 1979; Engel and Olinger, 1979; Clarke and Tsang, 1984) and anion-exchange resins (Oshima et al, 1983), an amino-bonded silica (Rajakyla and Paloposki, 1983; Oshima et al, 1983; Linden and Lawhead, 1975) or silica coated with an amino modifier in the eluent (Aitzetmuller, 1978). We have used these columns very successfully in our laboratories but the limitation of the ion-exchangers is the poor resolution of oligosaccharides. The amino-bonded phase currently used is very useful and even quite durable for sugar analysis if handled correctly (Rajakyla and Paloposki, 1983). Recently many authors have published excellent papers dealing with separation of glucose oligomers on the ODS-column (octadecyl silica, C18) using water as the eluent (Heyraud and Rinaudo, 1980; Cheetham et al, 1981; Verhaar et al, 1984). The elution order is that of increasing molecular weight. The reversed phase chromatography with pure water as the eluent offers two important advantages compared with ion-exchange or amino columns; a rigid stationary phase and cheap, non-toxic eluent. In addition, it is also a good alternative in identifying sugars in complex mixtures, especially when connected to a mass spectrometer as reported here.

A number of diketose dianhydrides are known that are formed by elimination of two molecules of water between two ketose units (Beynon et al, 1968). The D-fructose derivatives have been made by the action of strong acid on D-fructose. These components are also formed in fructose glucose processes causing losses and some other minor problems. There is a lack of information on how the analysis of difructose dianhydrides can be performed. Krol has tried paper chromatography (Krol, 1978a, 1978b) and C. Tsang (1985) has used columns of ion exchange resin in the calcium form to separate these

components. It seems that an effective ODS-column, with water as the eluent, is probably the best analysis method for difructose dianhydrides.

EXPERIMENTAL

The experiments were carried out using a Varian 5000 liquid chromatograph (Varian Aerograph, Walnut Creek, CA, U.S.A.) with a Knauer refractive index detector (Knauer, RI, type 98.00, Dr. Herbert Knauer Wissenschaftliche Gerate KG, Bad Homburg, F.R.G.).

The columns, stainless steel, 250 x 4.6 mm I.D., were packed in isopropanol slurry using methanol as pressurising solvent, with the following modified 5-micron silicas:

Column 1. Spherisorb S 5 ODS 2 (Phase Separations, Ltd., Queensferry, UK).

Column 2. Nucleosil 5 C18 (Macherey-Nagel, Duren, FRG).

Column 3. Vydac 201 HSB 5 Reverse Phase (The Separations Group, Hesperia, California, USA).

Column 4. Spherisorb S 5 C8 (Phase Separations, Ltd., Queensferry, UK).

Column 5. Spherisorb S 5 C6 (Phase Separations, Ltd., Queensferry, UK).

Column 6. Vydac 201 TPB 5 Reverse Phase (The Separations Group, Hesperia, California, USA).

Column 7. Spherisorb 5 C18 (300 A) (Phase Separations, Ltd., Queensferry, UK).

Column 8. Shandon PB 17 B, WP 300, C18 (Shandon Southern Products Limited, Cheshire, UK).

The pore size in the first five is about 100 A; in the rest, 300 A.

The columns were run under the following conditions: column temperatures 5-25° C, eluent water, flow rate 1.0 ml/min, injection amount 20 µl of 0.1% (w/v) solutions of samples.

Dried DE 42 corn syrup was obtained from Hayashibara Biochemical Labs. (Japan). The difructose dianhydrides were kindly donated by Dr. W. S. Charles Tsang (Sugar Processing Research, Inc., New Orleans, LA, USA) and all other sugars were commercially available materials.

The LC/MS runs were carried out at the application laboratory of S. N. Nermag (Paris, France).

RESULTS AND DISCUSSION

Influence of the Temperature on the Retention of Carbohydrates

The DE 42 corn syrup and some separate sugar samples were run on column 1 at three different column temperatures. Table 1 and Figure 1 show very clearly that lowering the temperature results in longer retention times and better resolution. On this particular column we could not obtain a single peak for each glucose oligomer, with no separation of anomeric forms, at temperature as high as 55° C, as has been reported previously (Vrantny et al, 1983).

Comparison of Sugar Retention on Three 5- μ m ODS-Columns

Chromatograms of the DE 42 corn syrup sample and the retention times of the sugars used in this work on columns 1, 2 and 3 are shown in Figure 2 and in Table 2, using 15° C as the column temperature. From these data it can be seen that column 1, packed with Spherisorb 5 ODS 2, is more suitable for these samples. Nucleosil C18 is also quite satisfactory, but the Vydac 201 HSB differs in selectivity from the other two. This may be due to the different types of silica produced by The Separations Group.

The Effect of the Length of the Alkyl Chain on the Retention Behavior of Carbohydrates

All the samples were run at 15° C on column 1, column 4 and column 5 phases manufactured by Phase Separations based on exactly the same silica. When the length of the alkyl chain is decreased, the retention times of the carbohydrates decrease except in the case of fructose, glucose and diheterolevulosan I, as can be seen in Figure 3 and in Table 3. The separation mechanism is probably due to hydrophobic interaction and when the hydrophobicity of the column material decreases (C18 > C8 > C6), the interaction between the column material and the saccharides decreases, which means lower retention times.

The Influence of Pore Diameter of Silica on Retention Behavior of Carbohydrates

When packings based on silica of 300 A were used, it was found that the retention times and the resolution of sugars were decreased significantly compared to the results obtained with 100 A C18-silica. This is illustrated in Figure 4, which shows the chromatograms of a sugar mixture, containing fructose,

Table 1. Effect of temperature on the retention times (min) of sugars using column 1.

Sugar	Retention time (min)		
	25°C	15°C	5°C
Fructose	2.50	2.55	2.53
Maltose	2.82	2.91	2.97
Sucrose	3.38	3.50	3.75
Panose	3.68	3.85	4.15
Stachyose	4.22	4.50	5.12
Raffinose	4.64	4.95	5.83
Diheterolevulosan I	3.25	3.36	3.46
Diheterolevulosan II	3.92	4.04	4.35
Difructose dianhydride I	4.16	4.36	4.77
Difructose dianhydride II	3.79	3.90	4.19
Difructose dianhydride III	3.81	4.00	4.23

Table 2. Comparison of carbohydrate retention times (min)
on three different ODS columns.

Sugar	Retention time (min)		
	Column 1	Column 2	Column 3
Fructose	2.55	2.97	3.09
Maltose	2.91	3.43	3.45
Sucrose	3.50	4.01	3.85
Panose	3.85	4.58	4.30
Stachyose	4.50	5.24	4.57
Raffinose	4.95	5.79	4.88
Diheterolevulosan I	3.36	4.38	3.85
Diheterolevulosan II	4.04	4.51	4.53
Difructose dianhydride I	4.36	5.30	4.82
Difructose dianhydride II	3.90	4.38	4.39
Difructose dianhydride III	4.00	4.61	4.65

Table 3. The effect of the alkyl chain length on retention of carbohydrates.

Sugar	Retention time (min)		
	Column 1	Column 4	Column 5
Fructose	2.55	2.64	2.66
Maltose	2.91	2.75	2.77
Sucrose	3.50	3.02	2.96
Panose	3.85	3.04	2.94
Stachyose	4.50	2.96	2.83
Raffinose	4.95	3.21	3.02
Diheterolevulosan I	3.36	3.08	3.38
Diheterolevulosan II	4.04	3.49	3.37
Difructose dianhydride I	4.36	3.59	3.48
Difructose dianhydride II	3.90	3.45	3.34
Difructose dianhydride III	4.00	3.53	3.50

maltose, sucrose, panose, stachyose and raffinose, run under the same conditions on columns 1, 6, 7 and 8.

The Analysis of Di-D-Fructose Dianhydrides by Reversed Phase Chromatography Using Water as the Eluent

We have tried to analyze di-D-fructose dianhydrides formed in the glucose-fructose process on several different HPLC-columns, without any particular success until reversed phase chromatography was used. Comparison of retention times with those of authentic standards permitted identification of these components as shown in Figure 5, which is typical of the chromatograms given by fructose mother solutions. Identification by this method is not reliable unless confirmed, e.g. by mass spectrometry.

Application of a Combined Liquid Chromatographic-Mass Spectrometric Instrument Using a Thermospray Interface

In the past few years more and more reports of the successful combination of LC and MS have been published. This is probably indicative of the improved performance of the instruments available today and the solution of problems which are not overcome by use of GC-MS. The application of the thermospray instrument in analysis of starch hydrolysate samples has been tested by coupling this to an ODS column, using water as the eluent, flow rate of 0.5 ml/min and post-column addition of chemical ionization agent 0.05 M NH_4^+ AC at flow rate 0.5 ml/min. TIC chromatograms of positive and negative ions are presented in Figure 6. The LC-MS negative ion spectra of glucose and maltose are shown in Figure 7. The spectra have not been completely interpreted but the following fragment ions can be assigned: m/z 179 ($\text{GLU}-\text{H}^+$), m/z 161

$(\text{GLU}-\text{H}^+ - \text{H}_2\text{O})$, m/z 143 ($\text{GLU}-\text{H}^+ - 2 \text{H}_2\text{O}$), m/z 215 ($\text{GLU}-\text{H}^+ + 2 \text{NH}_4^+$), m/z 221 ($\text{GLU} + 2 \text{H}^+ + \text{Ac}^-$)?, m/z 341 ($\text{DP2}-\text{H}^+$), m/z 377 ($\text{DP2}-\text{H}^+ + 2 \text{H}_2\text{O}$).

SUMMARY AND CONCLUSION

High performance liquid chromatography of carbohydrates on different types of reversed phase columns using refractive index detection and water as the eluent was studied. Comparisons between the ODS materials of some suppliers have been made and the influence of column temperature, the length of the alkyl chain bonded to the silica and the pore diameter of silica on the retention behavior of starch hydrolysates and some other sugar samples has been investigated. A method to determine di-D-fructose-dianhydrides, formed in the glucose-fructose process, on an ODS column is described.

It was concluded that decreasing the column temperature results in increased retention times and better resolution. Decreasing the length of the alkyl chain or use of silica with larger pore diameters decreases the resolution and retention times. It has also been shown that a combination of the chromatographic system described in this study and the modern thermospray quadrupole mass spectrometer works quite satisfactorily with the starch hydrolysate sample.

Reversed phase chromatography is a very good alternative to either ion exchange or amino columns for analysis of sugars. RP packings have the great advantage of separating oligosaccharides using pure water as the eluent. The separation can be carried out at room temperature, but by lowering the temperature it is possible to increase the resolution and retention of carbohydrates. By making the alkyl chain bonded to silica shorter or using silica with a larger pore diameter it is possible to change the retention behavior of oligosaccharides significantly.

ACKNOWLEDGEMENTS

I am grateful to Dr. W. S. Charles Tsang for kindly donating the samples of di-fructose anhydrides. I wish to acknowledge the personnel of the application laboratory of S. N. Nermag for the LC/MS runs. I am also grateful to Mr. Jerry Cunefare and the personnel of our lab and office for their great help.

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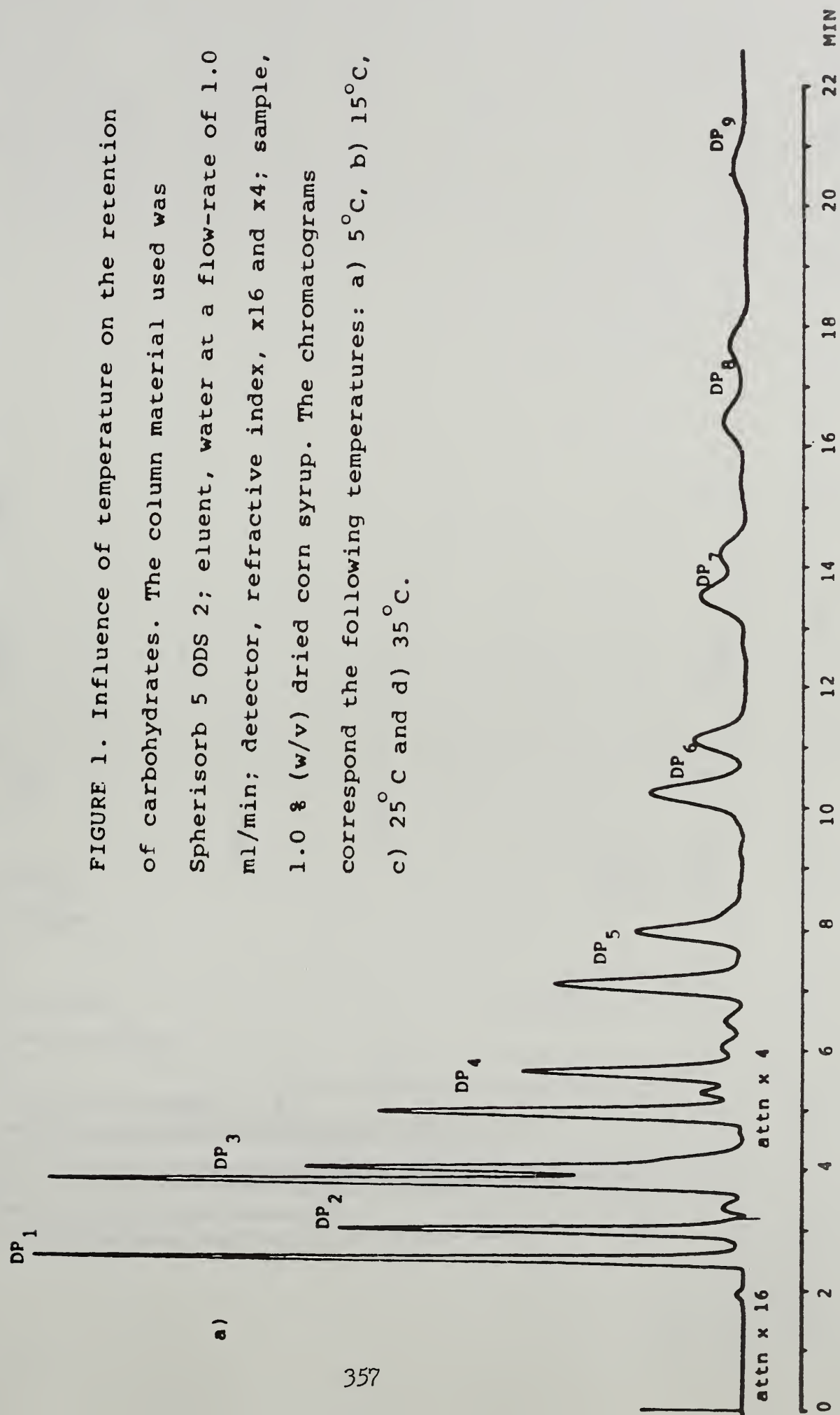


FIGURE 1. Influence of temperature on the retention of carbohydrates. The column material used was Spherisorb 5 ODS 2; eluent, water at a flow-rate of 1.0 ml/min; detector, refractive index, x16 and x4; sample, 1.0 % (w/v) dried corn syrup. The chromatograms correspond the following temperatures: a) 5°C, b) 15°C, c) 25°C and d) 35°C.

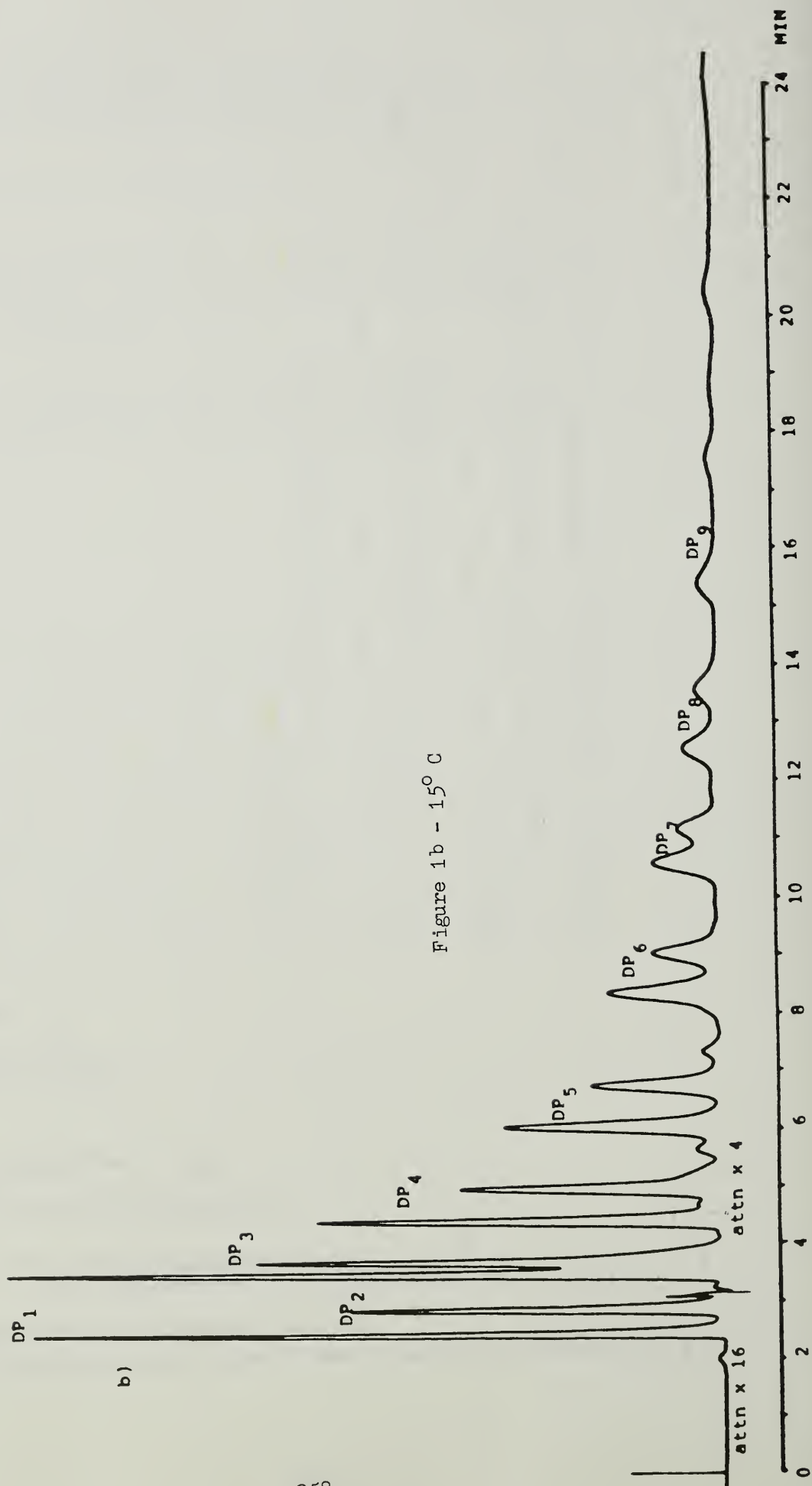
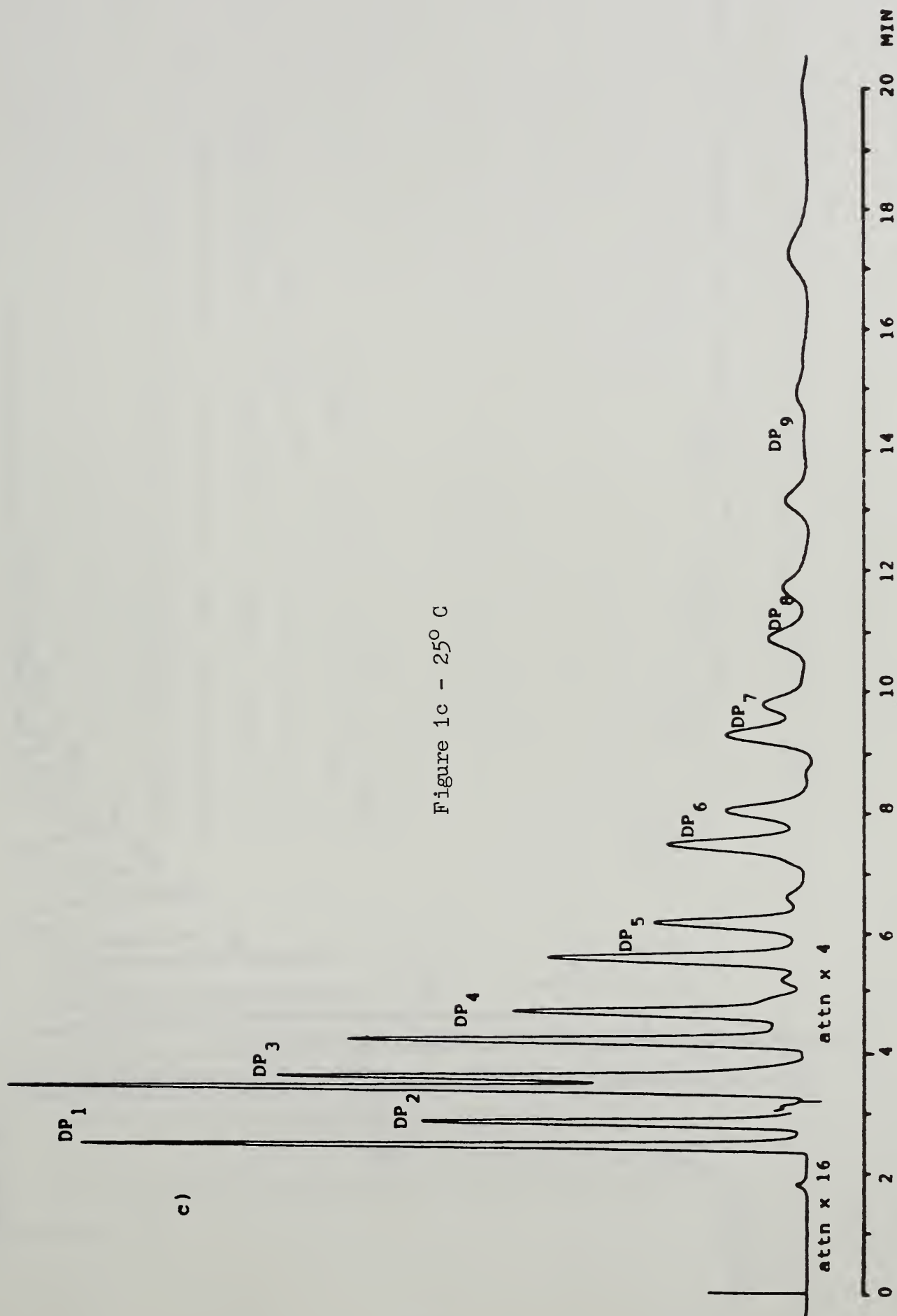
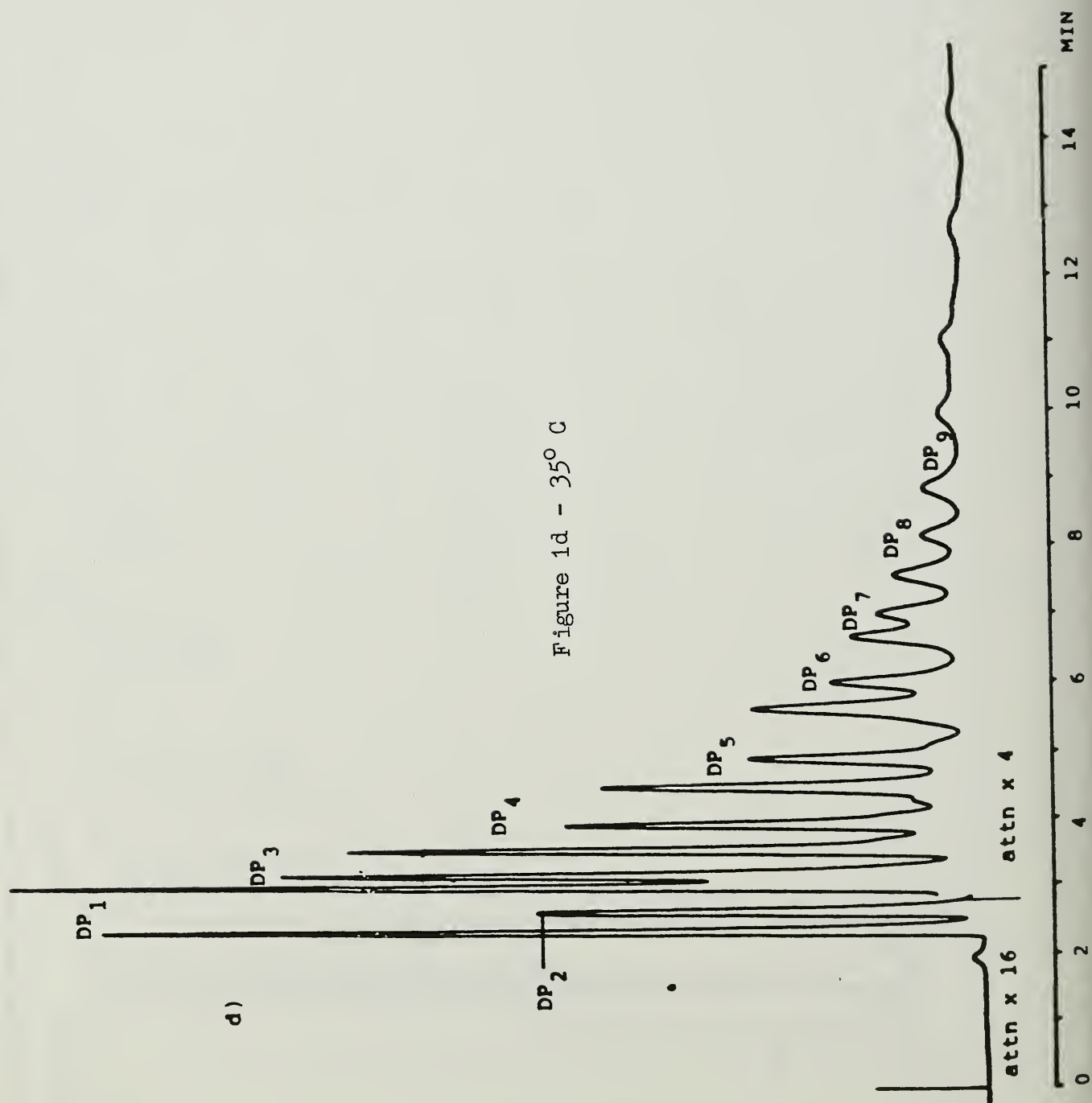


Figure 1b - 15° C





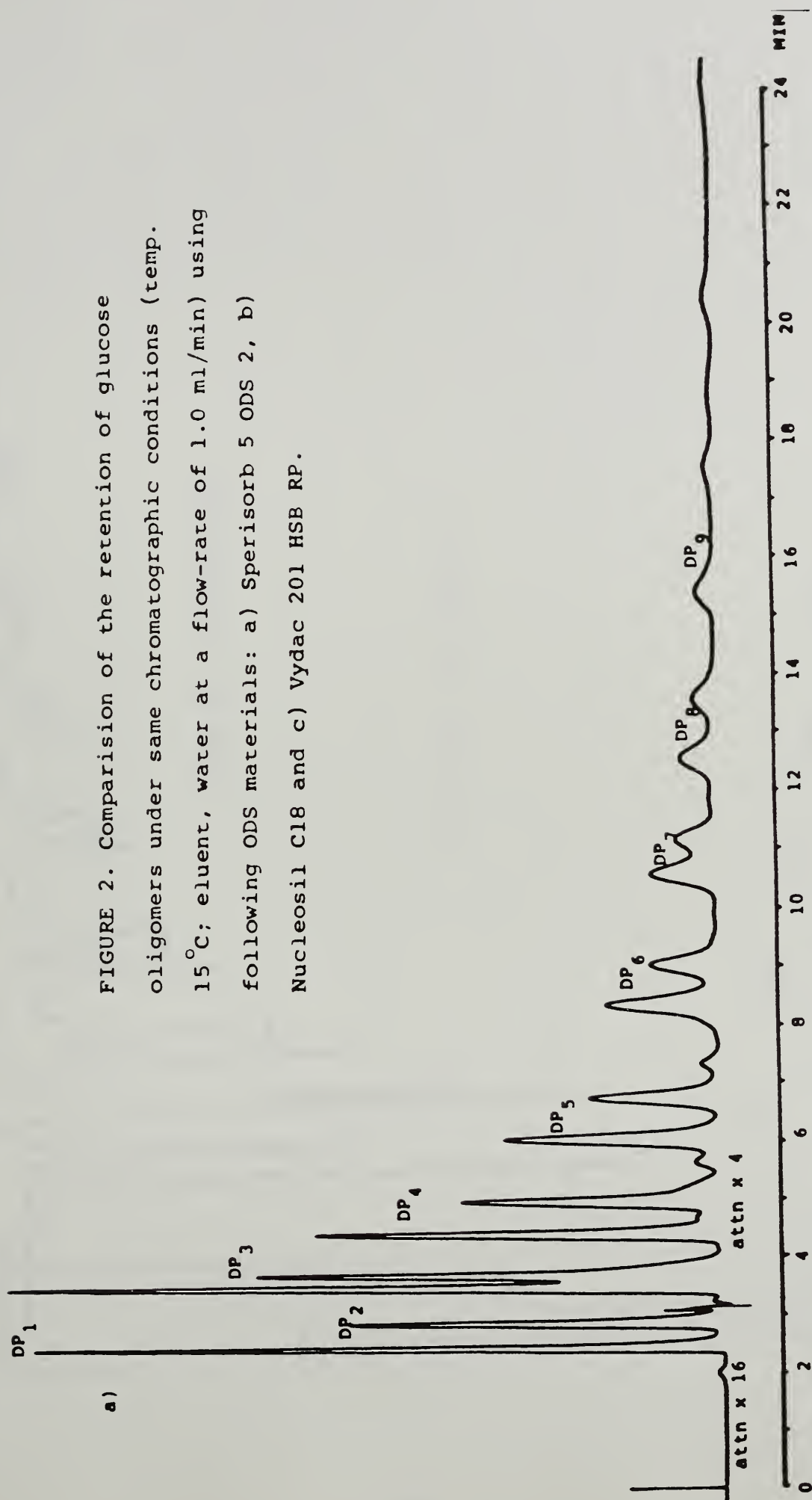


FIGURE 2. Comparison of the retention of glucose oligomers under same chromatographic conditions (temp. 15°C; eluent, water at a flow-rate of 1.0 ml/min) using following ODS materials: a) Sperisorb 5 ODS 2, b) Nucleosil C18 and c) Vydac 201 HSB RP.

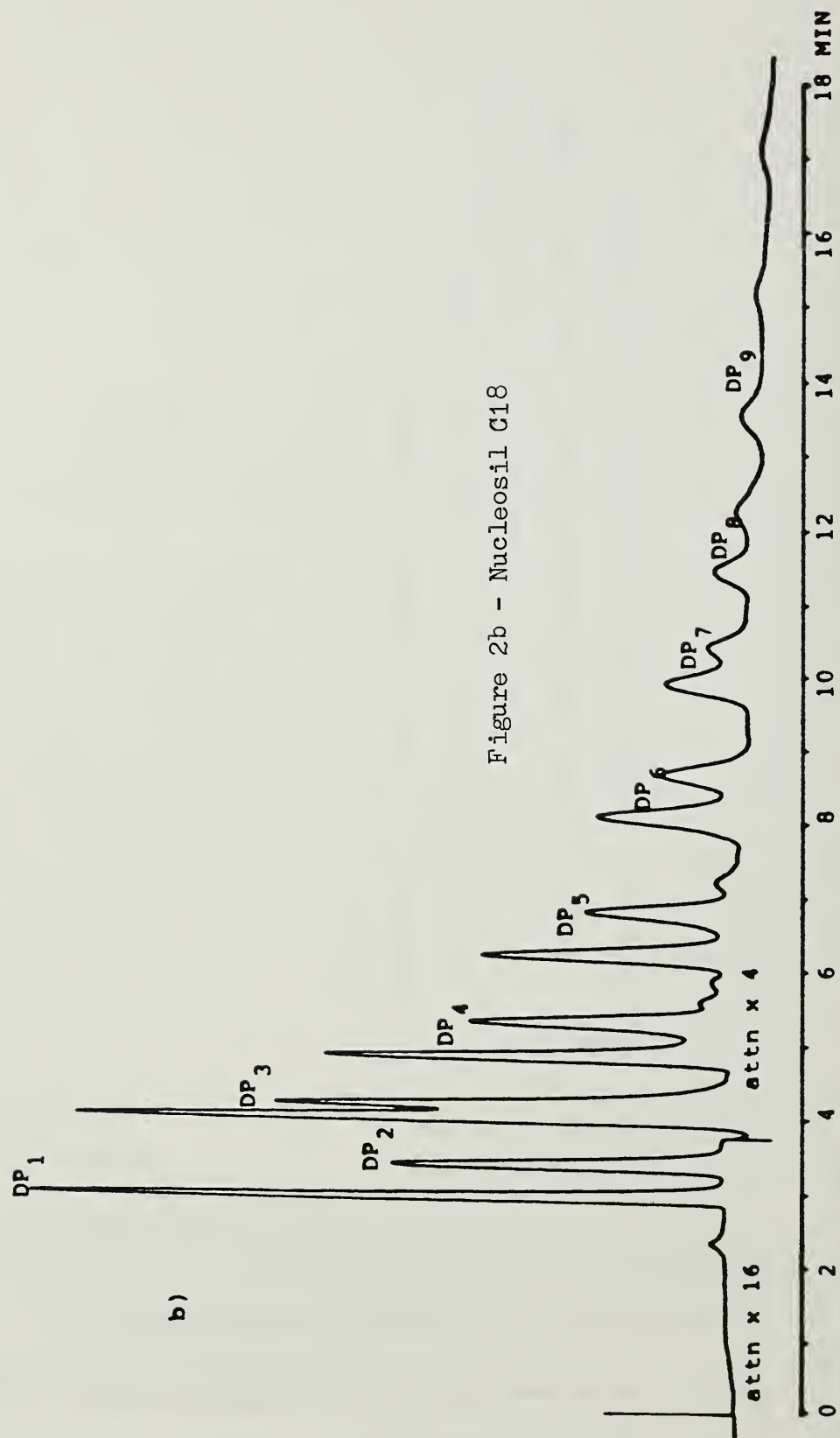


Figure 2b - Nucleosil C18

c)

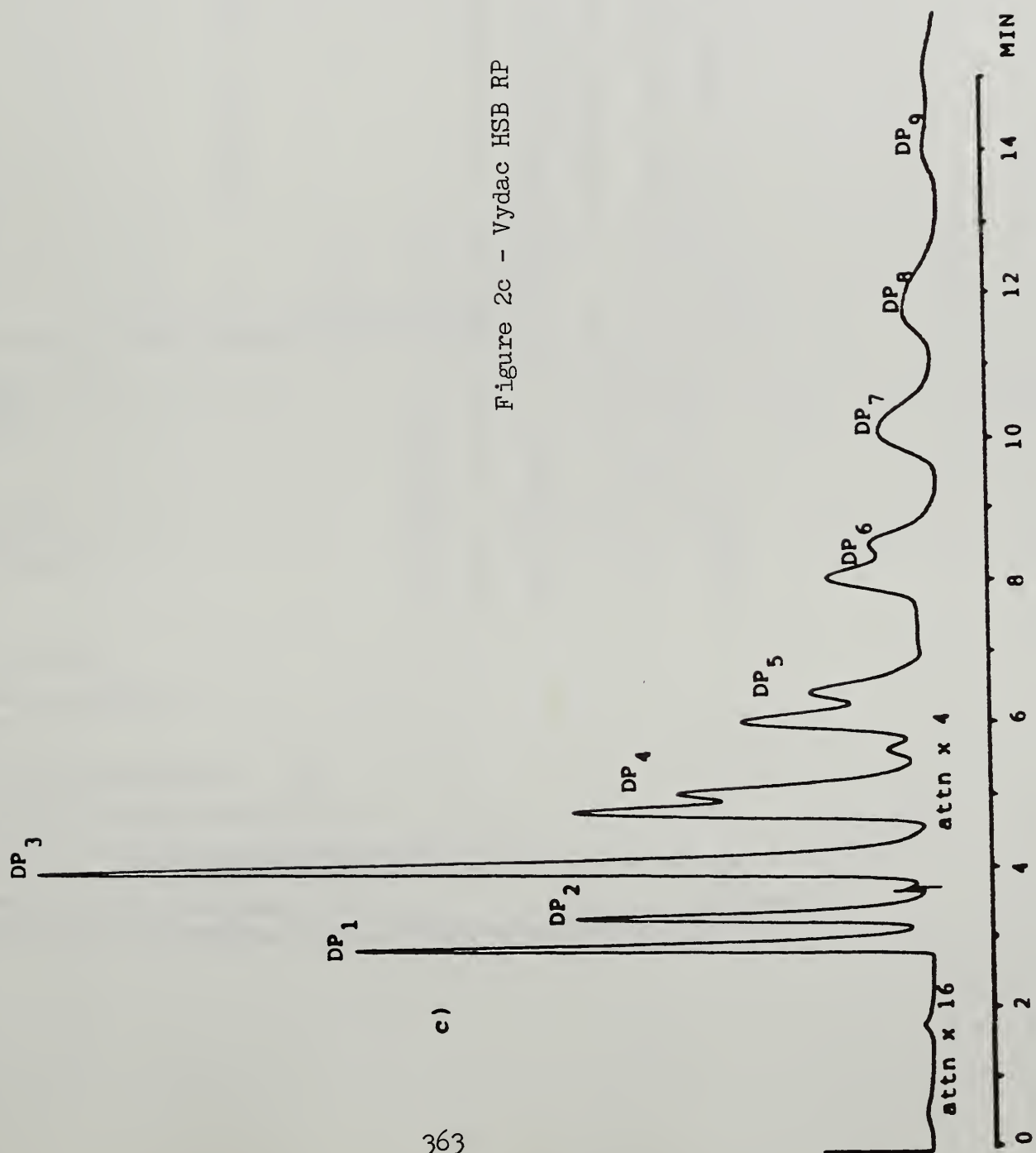
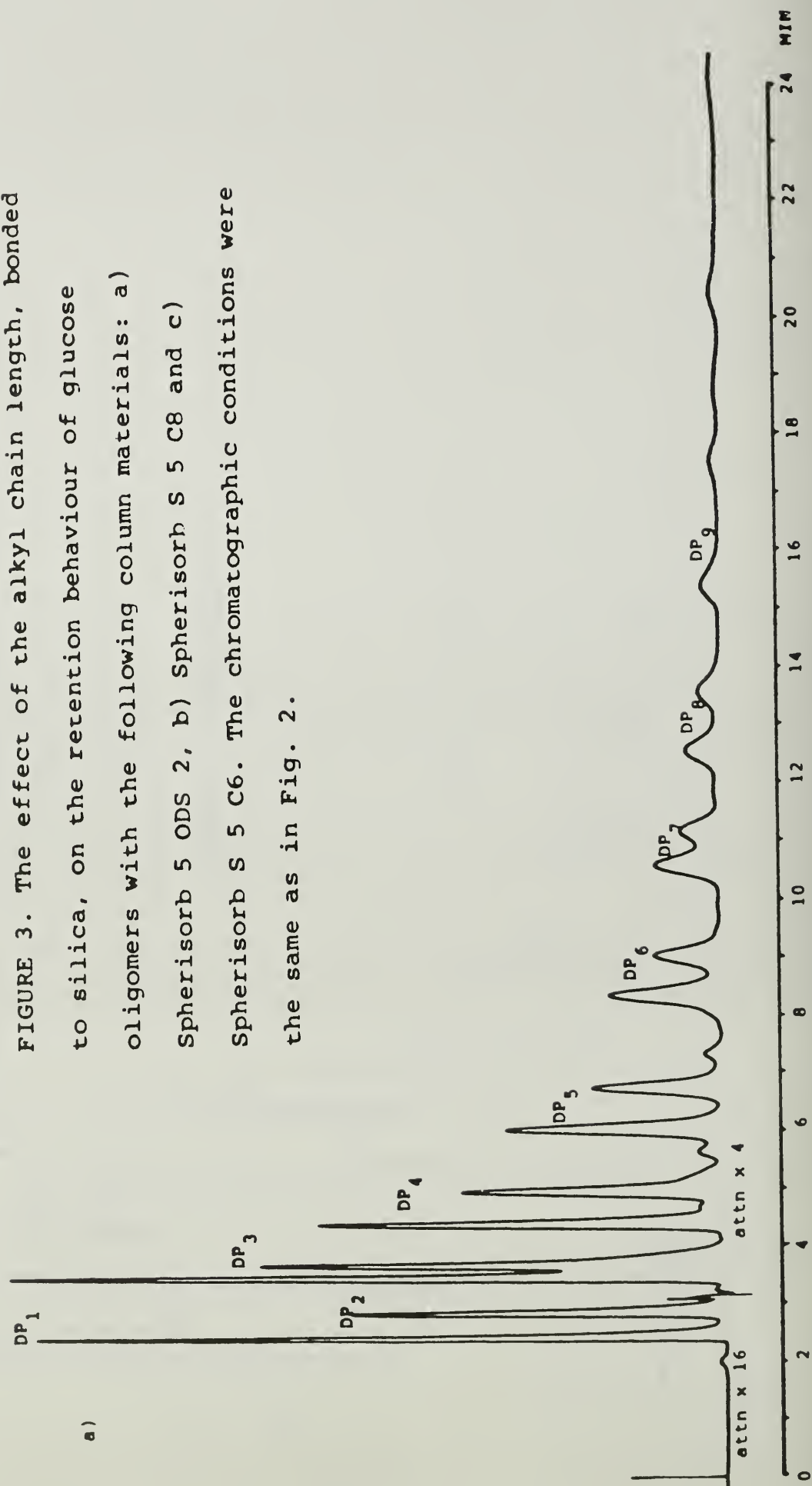


Figure 2c - Vydac HSB RP

FIGURE 3. The effect of the alkyl chain length, bonded to silica, on the retention behaviour of glucose oligomers with the following column materials: a) Spherisorb 5 ODS 2, b) Spherisorb S 5 C8 and c) Spherisorb S 5 C6. The chromatographic conditions were the same as in Fig. 2.



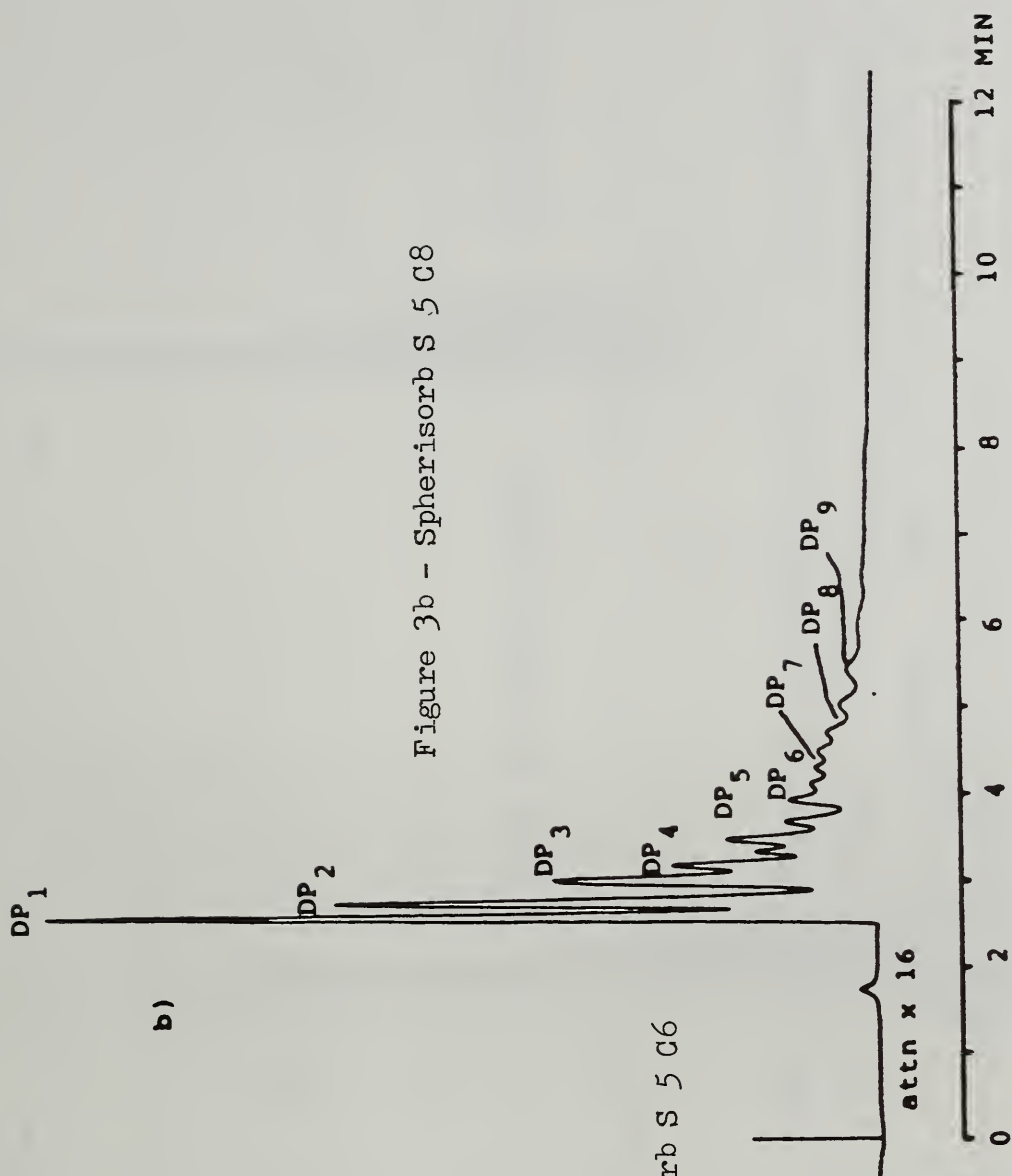


Figure 3b - Spherisorb S 5 C8

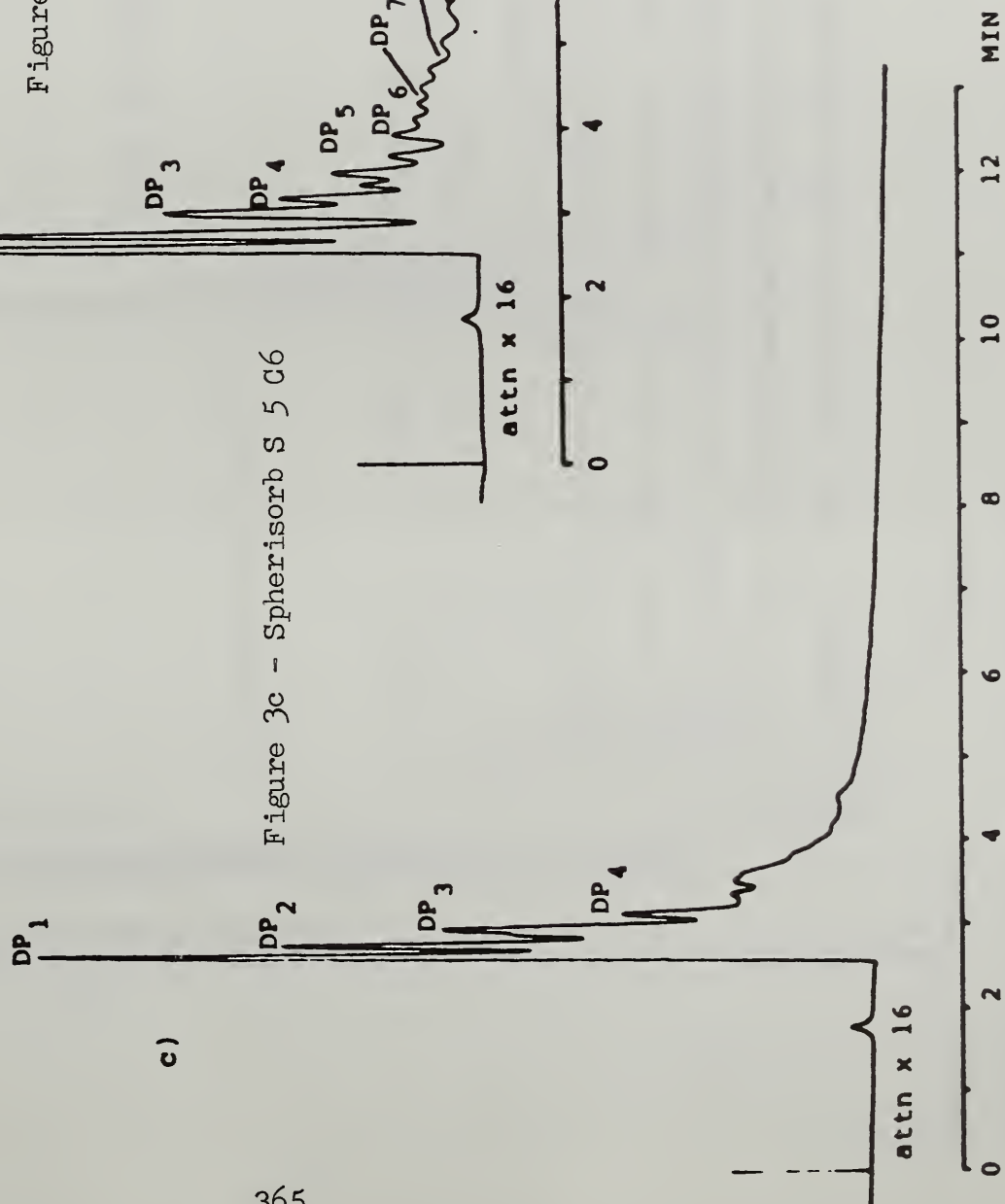


Figure 3c - Spherisorb S 5 C6

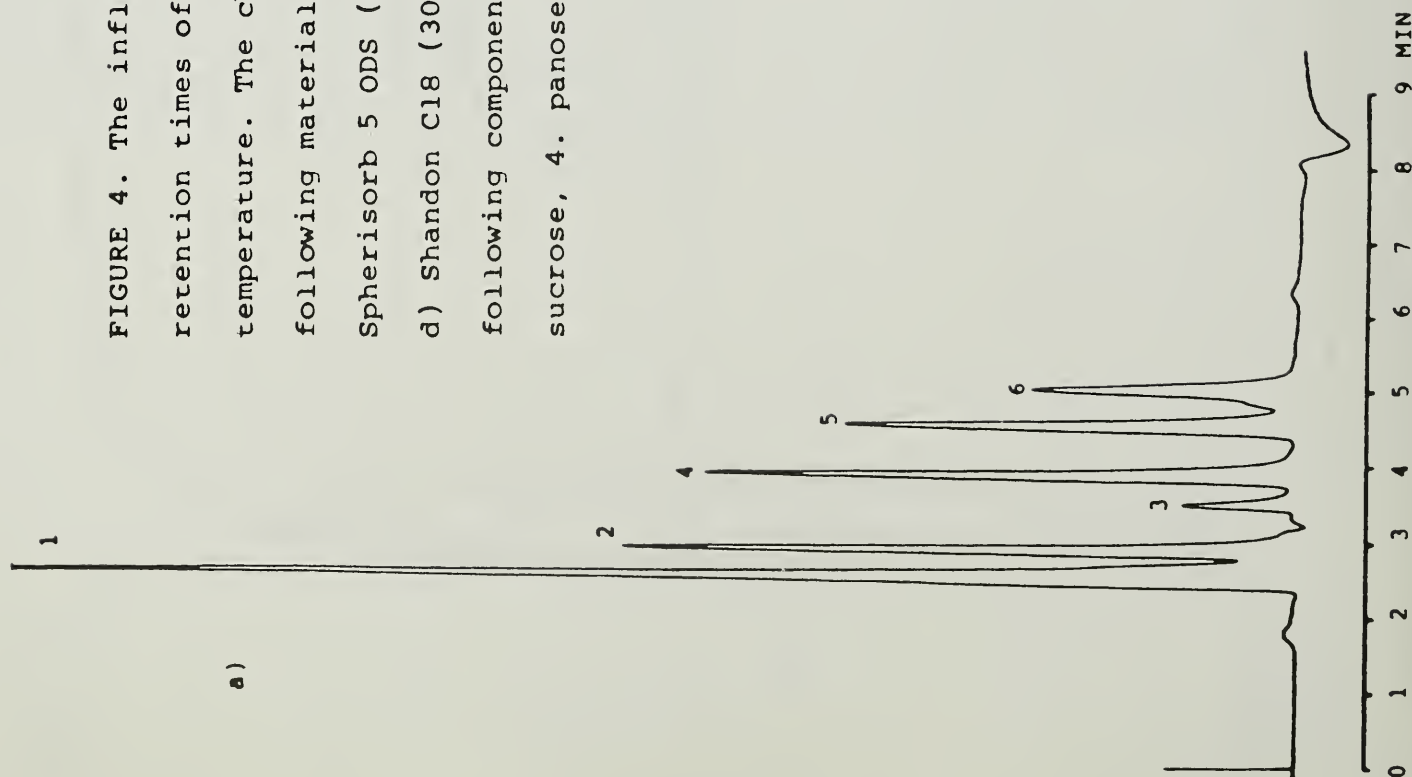


FIGURE 4. The influence of pore diameter of silica on retention times of saccharides at 15°C column temperature. The chromatograms were obtained on the following materials: a) Spherisorb 5 ODS 2 (100 Å), b) Spherisorb 5 ODS (300 Å), c) Vydac 201 TPB RP (300 Å), d) Shandon Cl8 (300 Å). Peaks correspond to the following components: 1. fructose, 2. maltose, 3. sucrose, 4. panose, 5. stachyose, 6. raffinose.

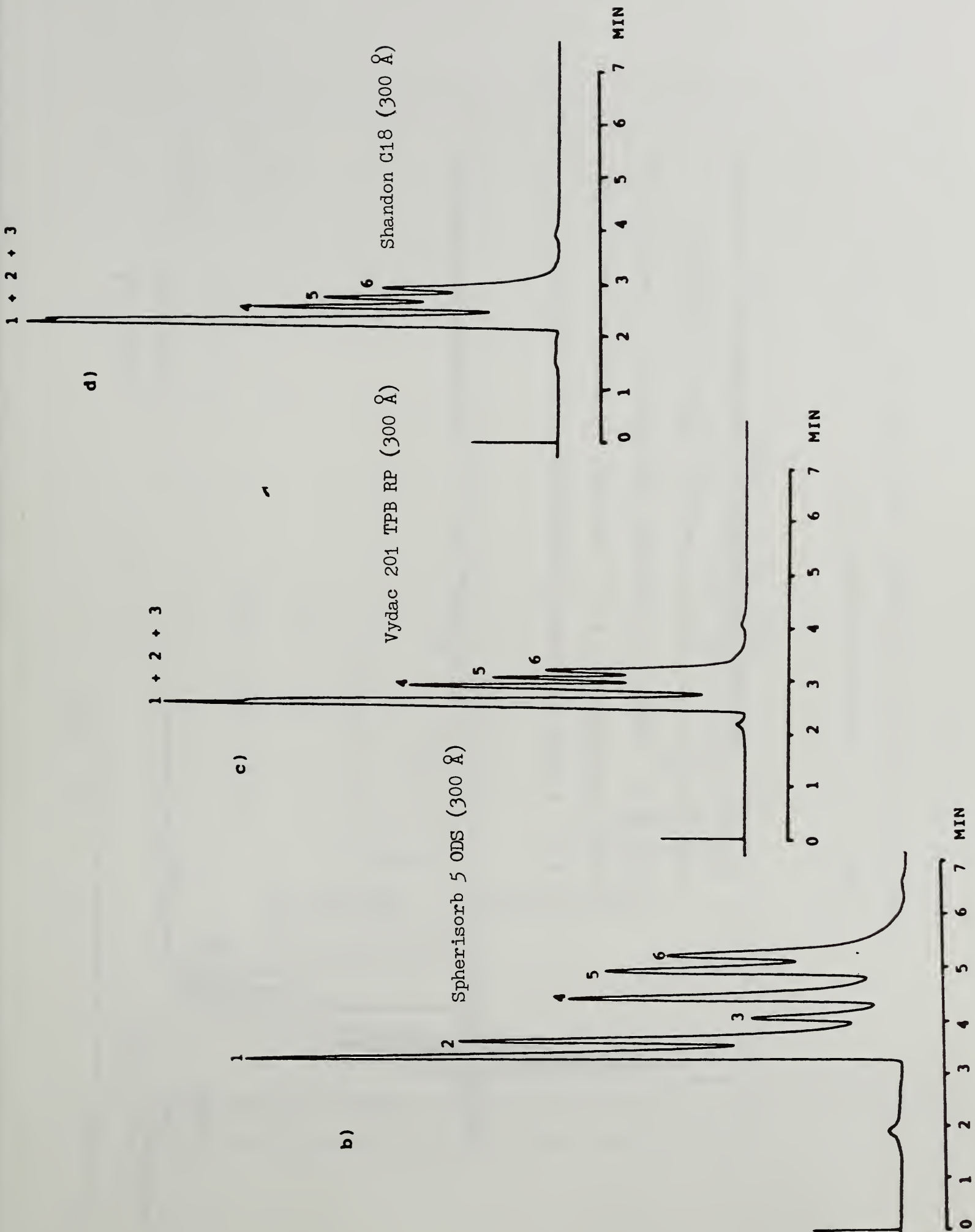
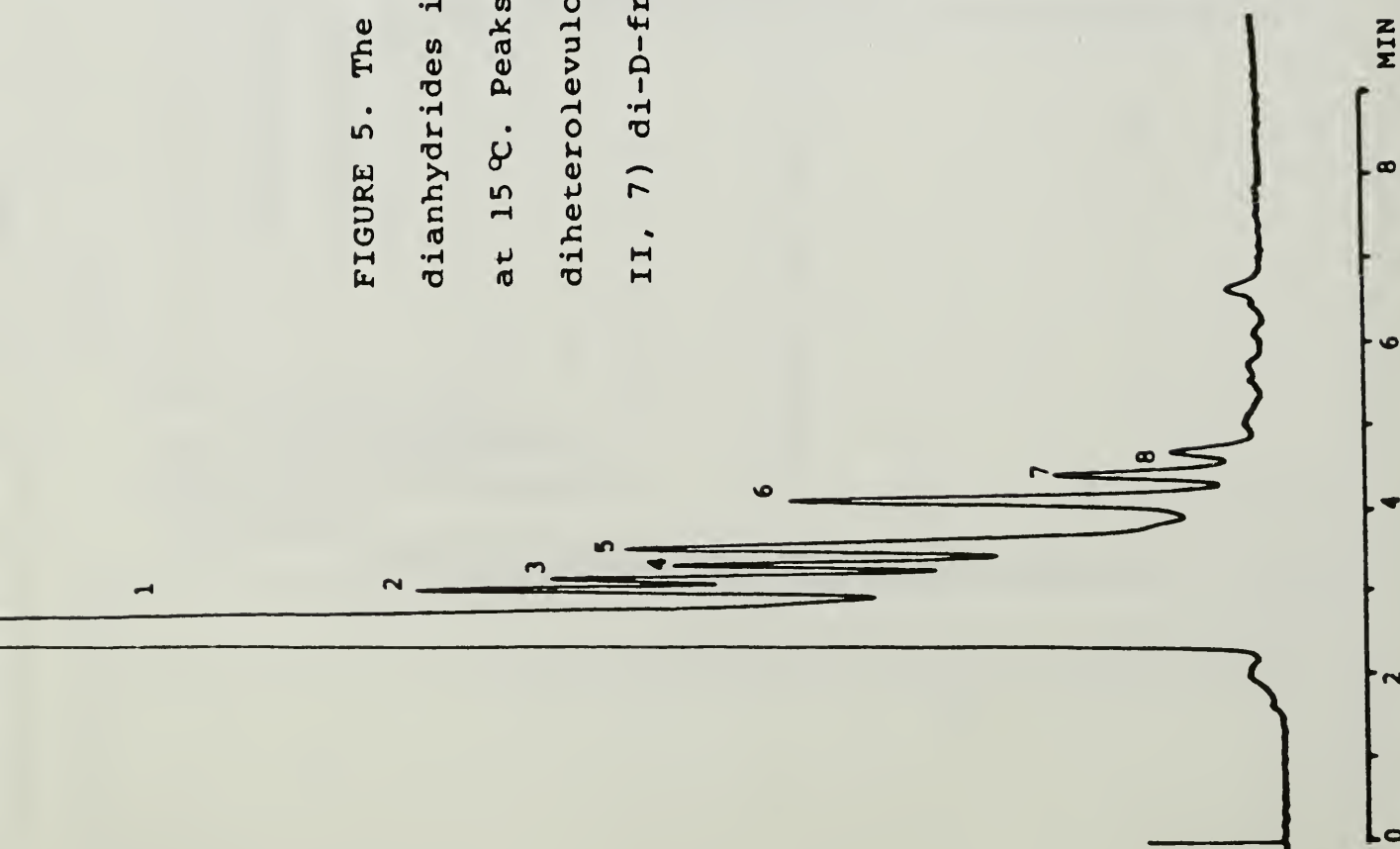


FIGURE 5. The determination of di-D-fructose dianhydrides in fructose mother liquor on Column 1 at 15°C. Peaks: 1) fructose, 2) unknown, 3) unknown, 4) diheterolevulosan I, 5) sucrose, 6) diheterolevulosan II, 7) di-D-fructose dianhydride I, 8) unknown.



19-APR-85
16-APR-85
16-APR-85

[100, 10]

MEMO/810AR
FILE A: FIN4
FILE B: FIN3

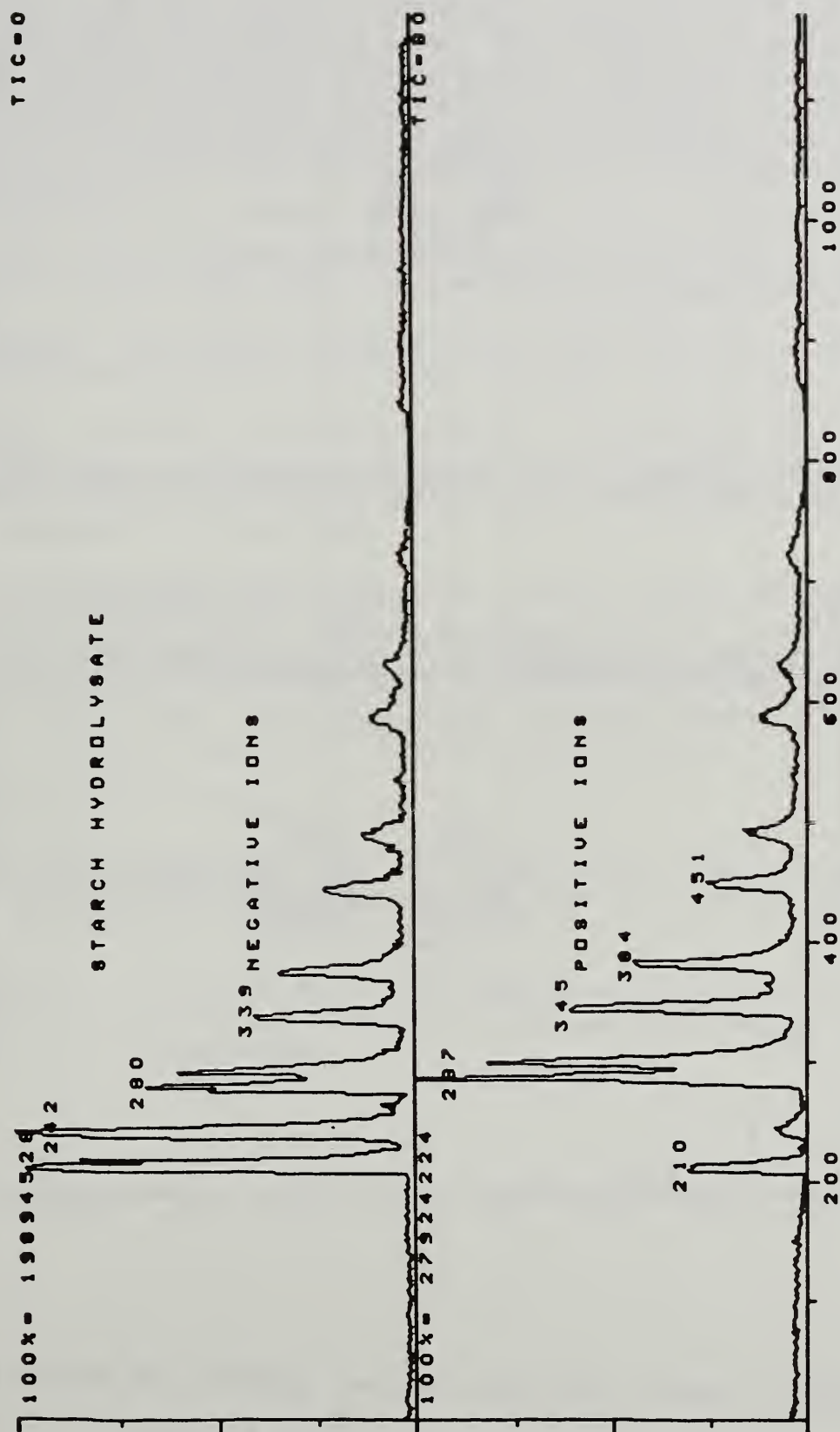
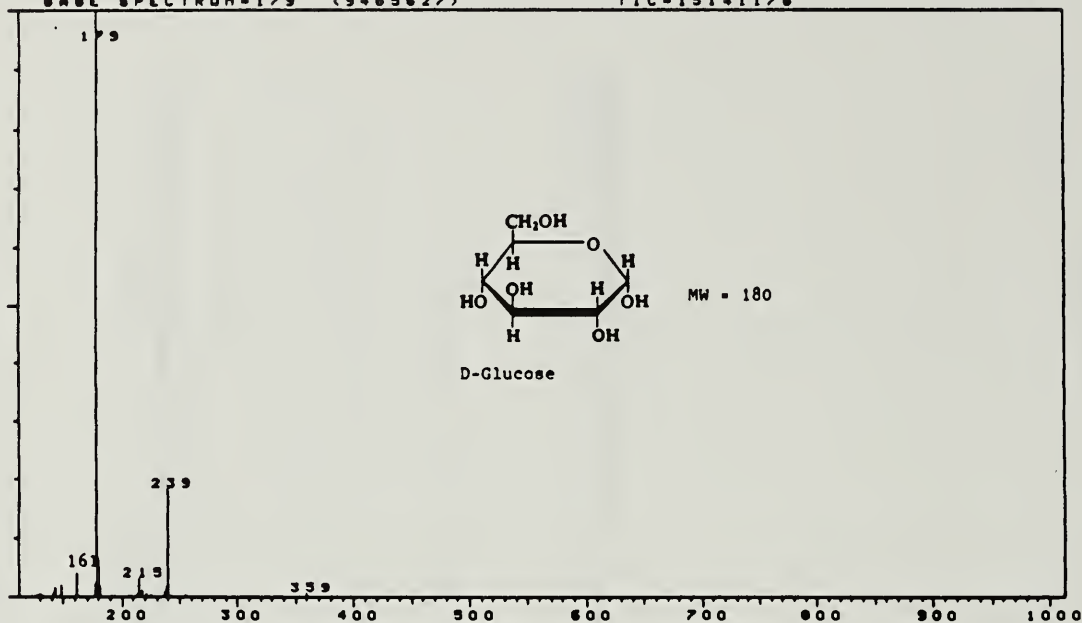


FIGURE 6. TIC chromatograms of positive and negative ions of the glucose oligomers. Conditions are mentioned in text.

STARCH HYDROLYSATE
100% = 9485627 RT = 04:30.6 SCAN = 2096220-1956204
BASE SPECTRUM = 179 (9485627) TIC = 19141178



STARCH HYDROLYSATE
100% = 3229508 RT = 05:08.4 SCAN = 2376240-2296233
BASE SPECTRUM = 179 (3229508) TIC = 14099166

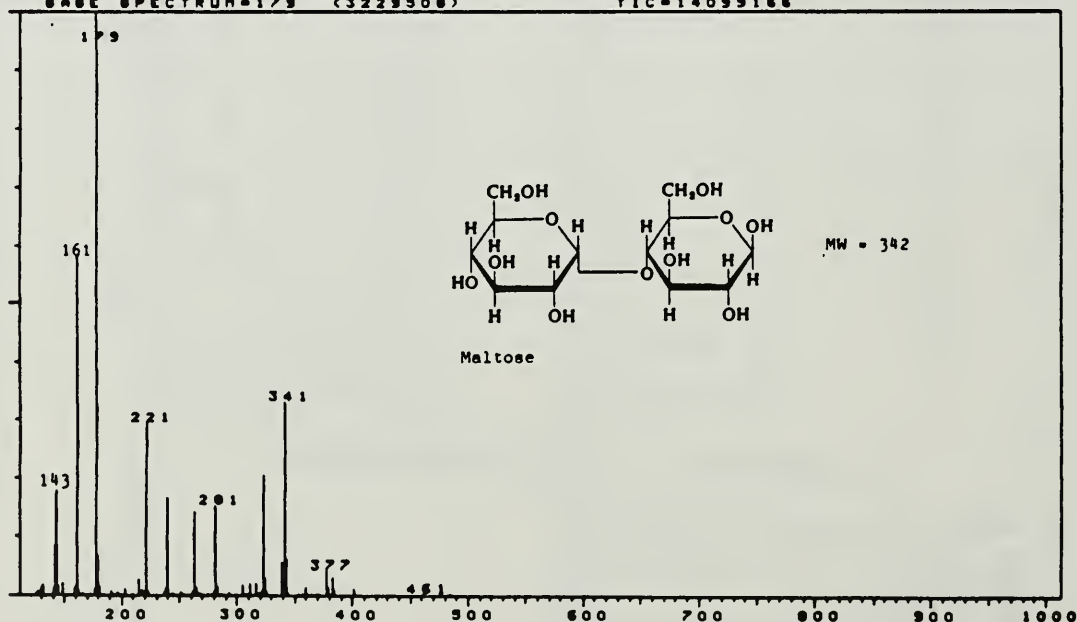


FIGURE 7. The LC MS negative-ion spectra of glucose and maltose. See the interpretation in text.

DISCUSSION

Richard Riffer, C and H Sugar - You asked early in your talk for comments on why glucose is more polar than other monosaccharides. I have a comment: glucose is the only aldohexose in which, disregarding the anomeric C-1, all the hydroxyls are equatorial. That means that they are more accessible and more able to participate in whatever is measuring the polarity. In other aldohexoses, some hydroxyls are axial to the plane of the molecule; these are more hindered and less accessible to the polarity measurement.

In the case of disaccharides, there is one fewer hydroxyl group per linkage, or per unit mass. That will also decrease polarity.

Rajakyla - Have you any explanation for the fact that on some systems fructose will elute before glucose?

Riffer - Fructose is different because a significant fraction doesn't have the hexagonal pyranose ring of glucose, and its hydroxyl groups are not exactly equatorial or axial. Fructose can't be compared in the same way.

T. Chadwick, Holly Sugar Corp. - In Figure 4, where you show several chromatograms, there is quite a difference in resolution between Figures 4b and 4d, even though they were obtained on packing material that apparently has the same porosity and the same C-18 alkyl groups. Obviously, there are other effects at work here. Have you had time to identify any of these?

Rajakyla - No, we have not.

Margaret A. Clarke, S.P.R.I. - Would you please comment on the practical processing applications for HPLC that you have developed for use within your company's plants.

Rajakyla - We analyze starch hydrolyzates and maltose syrups by HPLC. It is also possible to analyze molasses and detect raffinose and other trisaccharides.

HPLC ANALYSIS OF CARBOHYDRATES: COMPARISON OF DETECTORS AND EVALUATION OF RECENT DEVELOPMENTS

W. S. Charles Tsang, Margaret A. Clarke and Marta M. Valdes

Sugar Processing Research, Inc.

INTRODUCTION

High performance liquid chromatography (HPLC) has been widely used for analysis of individual sugars in foods (Jackson, 1980; Hurst and Martin, 1980; Richmond et al., 1981; Brobst and Scobell, 1981; Wade and Morris, 1982; Shaw and Wilson, 1983; Salvo et al., 1984). In our research laboratory, this technique is well established to analyze sugars and sugar related compounds (Clarke and Tsang, 1983; Tsang and Clarke, 1984). This report will describe several different aspects of the HPLC methods developed recently for sugar analysis.

PART I. COMPARISON OF DETECTORS

Because of the absence of a strongly absorbing UV chromophore, carbohydrates are usually detected by a differential refractometer. An advantage of refractive index detection (RI) is that it can be used directly without the need of derivatization. RI detection generally has limited sensitivity, however, and temperature fluctuations must be carefully controlled.

Common sugars have also been determined in food samples using a UV detector at 190-193 nm (Shaw and Wilson, 1983; Yang et al., 1981). Even though the UV detector is several times more sensitive than the RI detector, it is more prone to interferences. Mobile phase used in these analyses must be of high purity and the sample must be purified by ion exchange chromatography to eliminate interfering substances.

Recently, a discrepancy in the result of sugar analyses obtained among different laboratories using these two detectors led us to reinvestigate the feasibility of using UV absorption at 190 nm for detection of sugars. The two systems of detection for sugars, cane juices and molasses are compared.

With a UV detector, preliminary purification by ion exchange chromatography is absolutely required to obtain samples pure enough for analysis (Figure 1). For pure sugars, both detectors give clean chromatograms (Figure 2) and similar results.

The ratios of peak areas between RI and UV for individual sugars in standard solution are calculated and compared with the ratios obtained for several cane juice samples (Table 1). The ratios of RI peak area/UV peak area in samples should be near or equal to the ratios in standard if there are no interferences from impurities. If impurities are included in the peak observed by UV, then the sample peak ratio will be lower than the standard ratio; if impurities show up in the RI detection, the sample ratio will be higher than the standard. In the case of cane juices (Figure 3), comparable results are obtained from both detection systems (Table 1) for sucrose, but results for glucose and fructose are not comparable.

Table 1.--Ratio of RI peak area/UV peak area for sugars and cane juices.

	Sucrose	Glucose	Fructose
Standard	788*	700*	216*
(C.V.)	(1.36%)	(1.78%)	(2.49%)
Juice (Houma)	801	725	202
CP65-357	817	660	317
CP74-383	798	722	228

*Average of six determinations

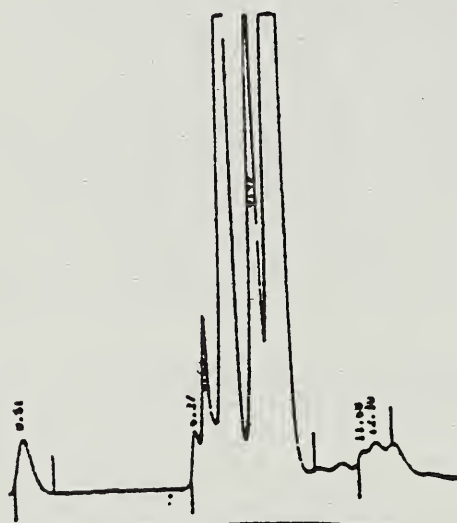
In the case of cane molasses analysis, ion exchange treatment of the sample will remove some but not all interfering substances. On the UV system, some non-sugar compounds give peaks which overlap with sucrose, glucose and fructose peaks (Figure 4b). There is no baseline separation between the sugar peaks. Interfering compounds will probably increase peak area of individual sugars. As a result, RI/UV ratio for cane molasses sample is lower than that for standard (Table 2), showing coelution of impurities on UV detection.

UV (190 nm)

a) Before ion-exchange
purification

b) After ion-exchange
purification

Cane
Juice



Cane
Molasses

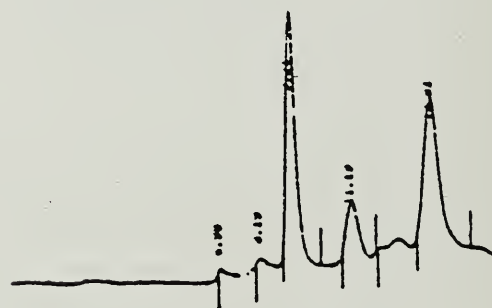
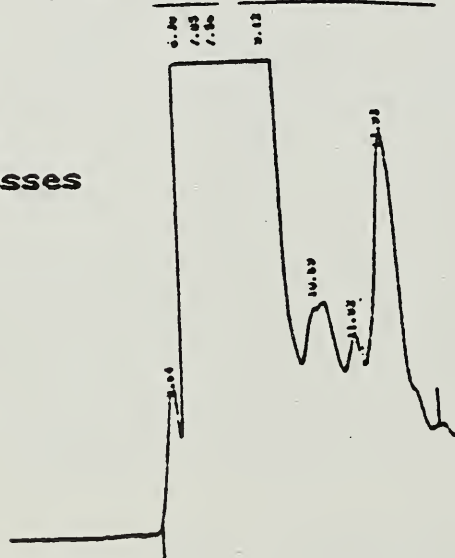


Figure 1.—Separation of sugars in cane juice and molasses on a Bio Rad HPX-87C column 30 cm long at a flow rate of 0.6 ml/min and 85° C. (a) Sample purified through Millipore membrane filtration; (b) sample purified by Millipore filtration followed by deashing cartridge.

Sugar standard

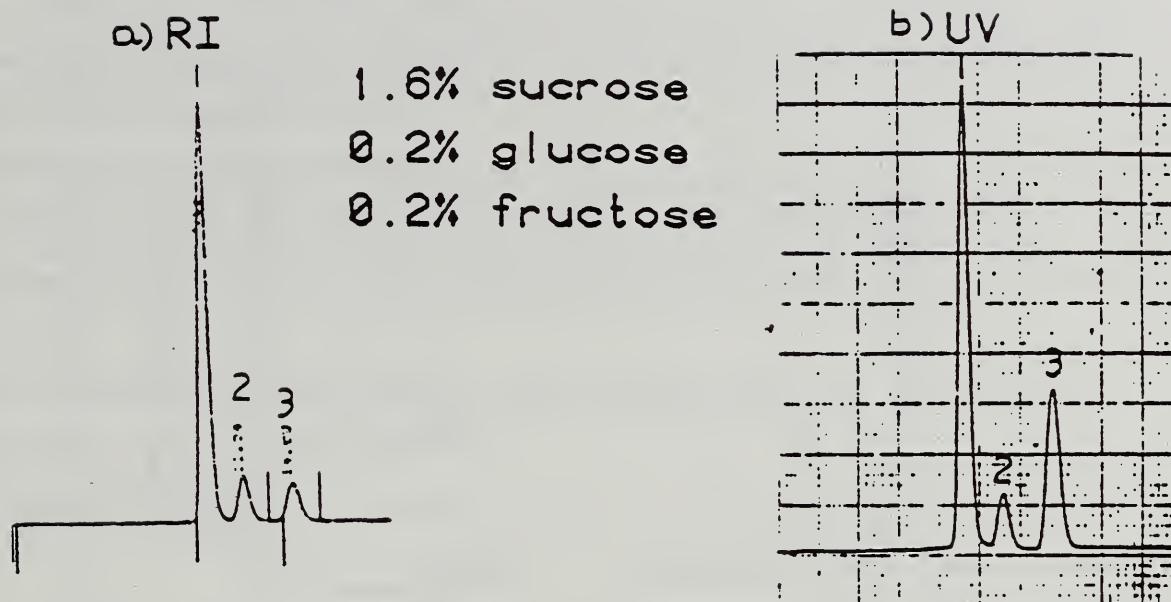


Figure 2.—Separation of standards on a Bio Rad HPX-87C column 30 cm long at a flow rate of 0.6 ml/min and 85° C. Peak identities: 1, sucrose; 2, glucose; 3, fructose. (a) RI detector; (b) UV detector at 190 nm.

Cane Juice (CP 74-383)

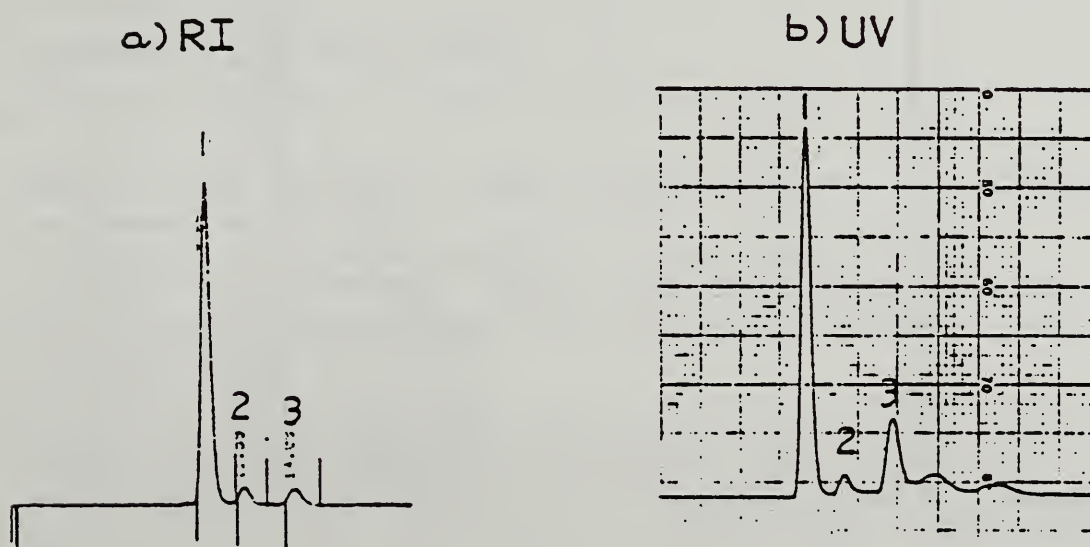


Figure 3.—Separation of sugars in cane juice on a HPX-87C column 30 cm long at a flow rate of 0.6 ml/min and 85° C. Peak identities: 1, sucrose; 2, glucose; 3, fructose. (a) RI detector; (b) UV detector at 190 nm.

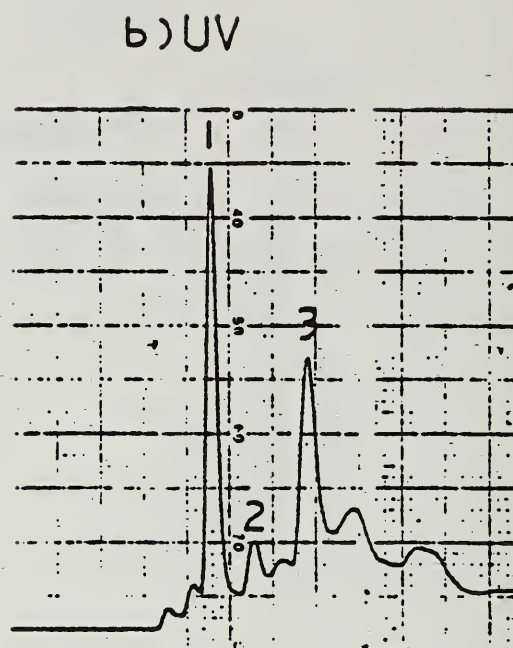
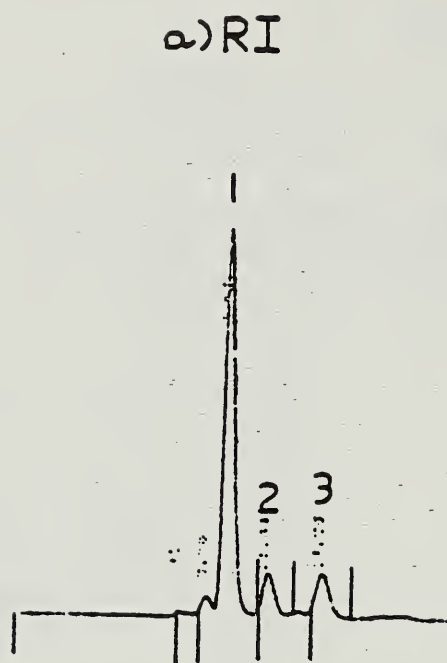


Figure 4.—Separation of sugars in cane molasses on a HPX-87C column 30 cm long at a flow rate of 0.6 ml/min and 85° C. Peak identities: 1, sucrose; 2, glucose; 3, fructose. (a) RI detector; (b) UV detector at 190 nm.

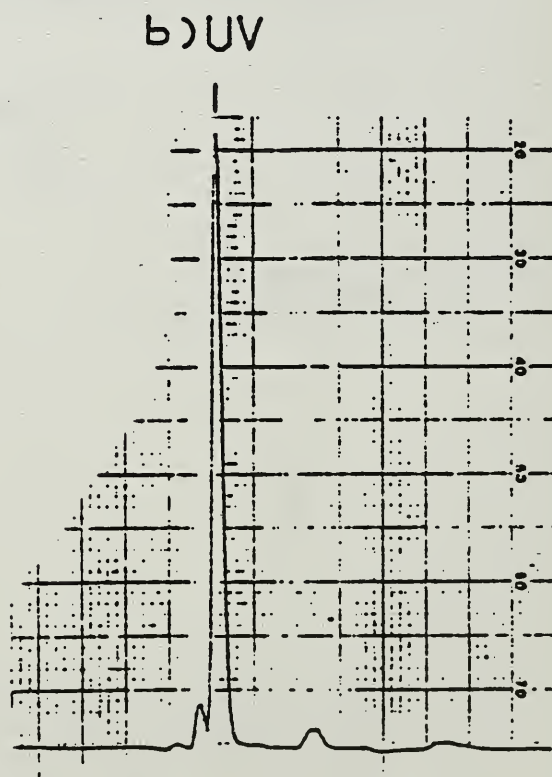


Figure 5.—Separation of sucrose in beet molasses on a HPX-87C column 30 cm long at a flow rate of 0.6 ml/min and 85° C. Peak identity: 1, sucrose. (a) RI detector; (b) UV detector at 190 nm.

Thus for low purity samples, results may not be the same by UV detection as by RI detection. UV analysis will probably show higher quantities of sugars. RI detection will give a more accurate analysis.

In beet molasses (Figure 5), where there is very little glucose or fructose, and sucrose is the main subject of analysis, UV detection gives results more like that of RI detection than in cane molasses analysis (Table 2).

Table 2.--Ratio of RI peak area/UV peak area for sugars and molasses.

	Sucrose	Glucose	Fructose
Standard	835*	770*	223*
A-Refinery molasses	746	516	130
B-Beet molasses	854	---	---
C-Cane factory molasses	743	778	127
D-Cane factory molasses	758	542	144

*Average of 3 determinations

PART 2. FAST ANALYSIS OF CANE JUICES AND OTHER SUGAR PRODUCTS

Carbohydrates can be separated on several types of HPLC columns. The three most commonly used types are amino-bonded silica, alkyl reverse phase and strong cation exchange (resin based) columns.

The use of reverse phase columns for the separation of invert sugars, sucrose (Rajakyla and Paloposki, 1983; Palla, 1981) and oligosaccharides (McGinnis et al., 1986; Rajakyla, 1986; Vratny et al., 1983; Cheetham and Sirimanne, 1981) has been reported. A recently developed analytical system was used for fast analysis of cane juices in a S.P.R.I. trial at a sugar factory. The column used in the radial compression system is

a Resolve C18, 5 μ Radial Pak cartridge with a precolumn of Resolve C18 Guard-Pak insert. Table 3 shows conditions of analysis for cane juice. Analyses shown are for sucrose and invert; glucose and fructose are not separated (Figure 6a). The short run time (5-6 minutes) is very appropriate for routine juice analysis, where sucrose and total invert are often the most useful analyses. The short radial column used in this method is cheaper than the steel column and water is used as the eluent, keeping down the cost of analysis.

Table 3.--HPLC conditions for fast analysis of cane juice.

Column:	Waters Resolve C18, 5 μ Radial Pak Cartridge with radial compression system
Guard Column:	Resolve C18 Guard-Pak Precolumn Insert
Mobile Phase:	Water
Temp.:	Ambient
Flow Rate:	0.7 ml/min
Detector:	RI
Time for analysis:	5-6 min.

Comparison of this system with the cation exchange column in calcium form (HPX 87C), as used by S.P.R.I. for many years, shows very good duplication on sugar results. For example, an average of 15.30% of sucrose on the C18 Radial Pak and 15.28 on the HPX-87C is obtained for a cane juice sample (Table 4). The invert level determined by both columns is very close (0.76 vs. 0.75).

Cane Juice

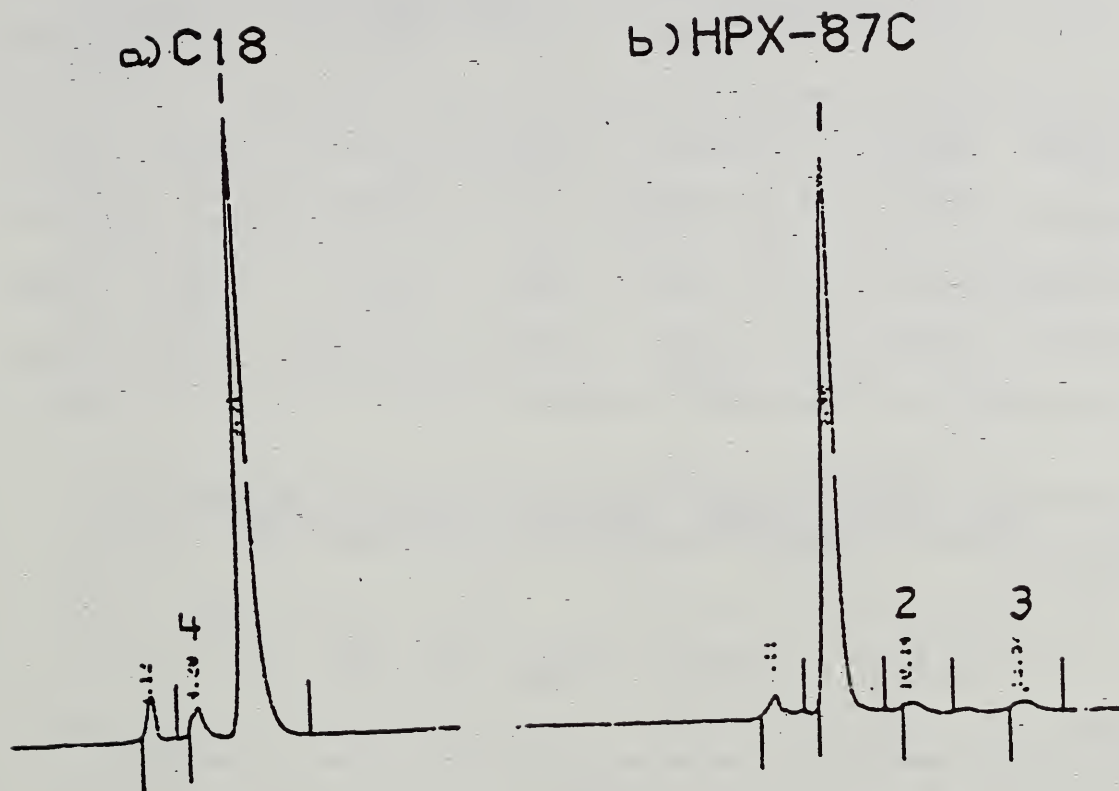


Figure 6.—Typical chromatograms of cane juice on (a) C18 column; (b) HPX-87C column. Peak identities: 1, sucrose; 2, glucose; 3, fructose; 4, invert.

Table 4.--Comparison of results obtained from two different systems.

	Cane Juice		Lemon-Lime Beverage		Syrup	
	C18	HPX-87C	C18	HPX-87C	C18	HPX-87C
% Sucrose	15.30	15.28	9.39	9.34	60.32	60.73
% Glucose	---	0.32	---	0.38	---	2.08
% Fructose	---	0.43	---	0.38	---	1.80
% Invert	0.76	0.75	0.75	0.76	3.37	3.88

The precision of the analyses has been determined as shown in Table 5, where "n" represents the number of injections measured.

Table 5.--Precision of sugar analysis.

Sugar	n	C.V.*	C.V.
		Column C18	Column HPX-87C
Sucrose	9	0.58%	0.28%
Glucose	9	--	1.40%
Fructose	9	--	1.52%
Reducing Sugars	9	1.29%	--

C.V. = coefficient of variation

During the field trial, a considerable amount of damaged cane was processed. HPLC results were useful in indicating high levels of invert in incoming juice.

PART 3. DIRECT DETERMINATION OF INVERT IN RAW SUGARS

The direct determination of invert in raw sugars and high purity syrup has been hampered by several factors: the lack of a suitable column for such separation, the inability to detect the sugars at low levels and the disparity in concentration between sucrose and invert sugar (99:1) in raw sugar.

Recently developed post-column derivatization methods such as tetrazolium blue (Betteridge et al., 1984; Wnukowski, 1984; Wight and van Niekerk, 1983; D'Amboise et al., 1980), 2-cyanoacetamide (van den Berg et al., 1984; Honda et al., 1984; Schlabach and Robinson, 1983), copper (II) ammonia complexes (Leonard et al., 1984; Grimble et al., 1983) and p-amino benzoic acid hydrazide (Vratny et al., 1985) greatly improved sensitivity and selectivity for reducing sugars. These methods, however, require elaborate equipment and chemicals.

A direct and rapid analysis of invert in raw sugar is attempted using recently developed Waters Nova-Pak C18 columns. The use of highly efficient 4 μ packing material should lead to an increase in overall resolution. Since the invert peak emerges first, it will not be overwhelmed by the large sucrose peak which emerges after the invert (Figure 7).

The precision of the analyses is shown in Table 6. The level of inverts in three different raw sugars as determined by this method at three different concentrations (0.5%, 1% and 2%) is tabulated in Table 7. It is suggested that 1% raw sugar solutions be used because they give results which are closer to the GC values (Table 7). A separate determination (0.25%, raw sugar) for sucrose is necessary because higher concentration will lead to sucrose overload on the columns and false readings.

Nova-Pak C18 Column

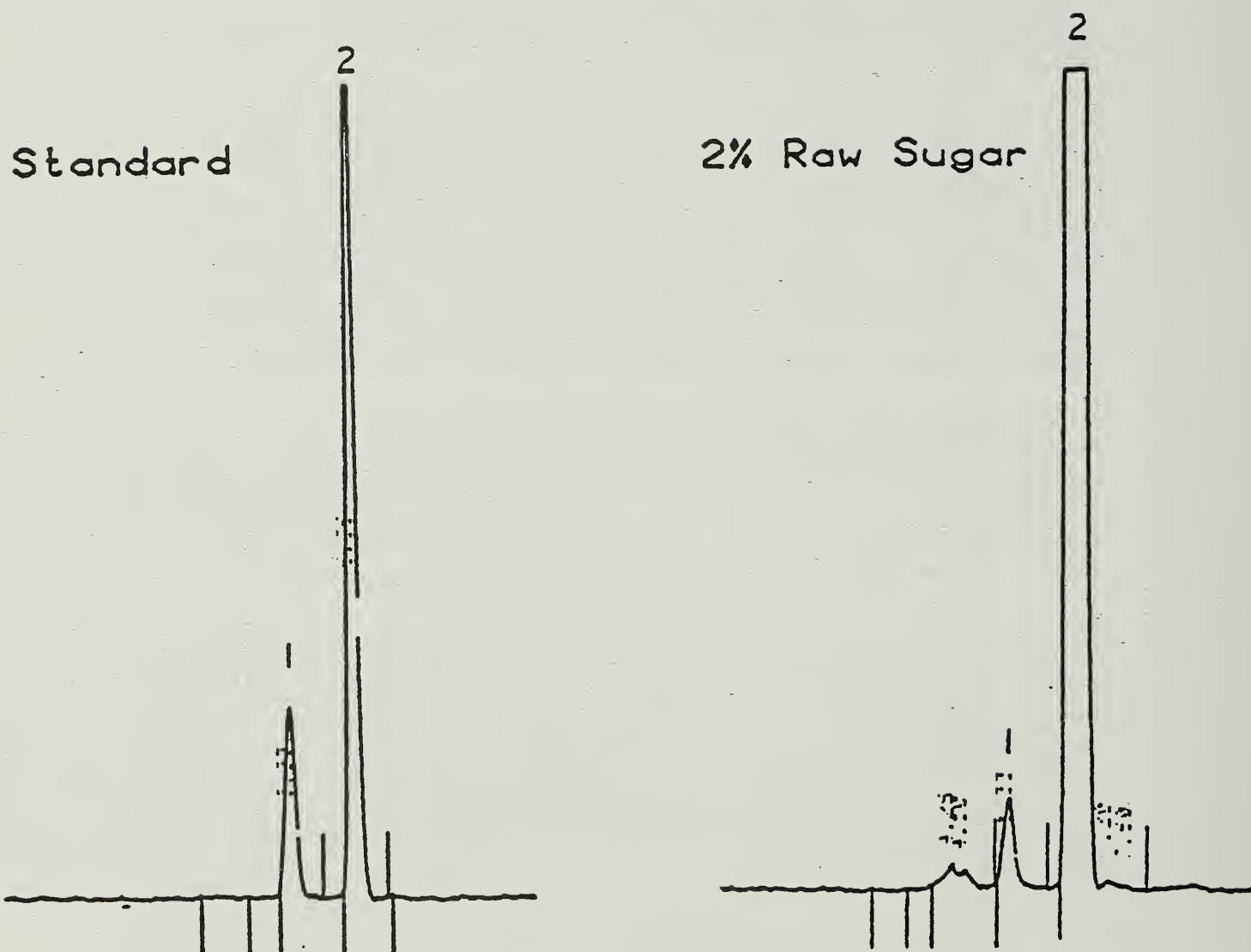


Figure 7.—Determination of invert in raw sugar by C18 Nova-Pak Column. Peak identities: 1, invert; 2, sucrose.

Table 6.--Precision of sugar analysis using
Nova-Pak Column.

Sugar	n	C.V.
Invert	9	0.9%
Sucrose	9	0.49%

Column: 2 Nova-Pak C18 steel columns
(3.9 mm x 15 cm)

Solvent: Water. Flow rate: 0.4 ml/min.

Detector: RI, 8X

Table 7.--Level of inverts in raw sugars.

Raw Sugar	Haiti	% of Invert Ecuador	Philippines	% of Sucrose (Philippines)
0.25%	--	--	--	97.52
0.5%	0.87	0.67	0.79	95
1%	0.83	0.71	0.70	89.06
2%	0.72	0.70	0.65	60.5
G.C.	0.829	0.806	0.717	--

A high sensitivity Waters 410 differential refractometer has also been used in this study (Figure 8). This RI detector is sensitive enough to be able to detect sub-microgram levels of sugar. This equipment is being studied further and indicates great potential for future work.

Recently, direct separation of carbohydrates by anion-exchange column with highly alkaline eluents has been reported (Rocklin and Pohl, 1983). This method provides a powerful new tool in

2 Nova-Pak C18 Steel Column +
Waters 410 Differential Refractometer (high sensitivity)

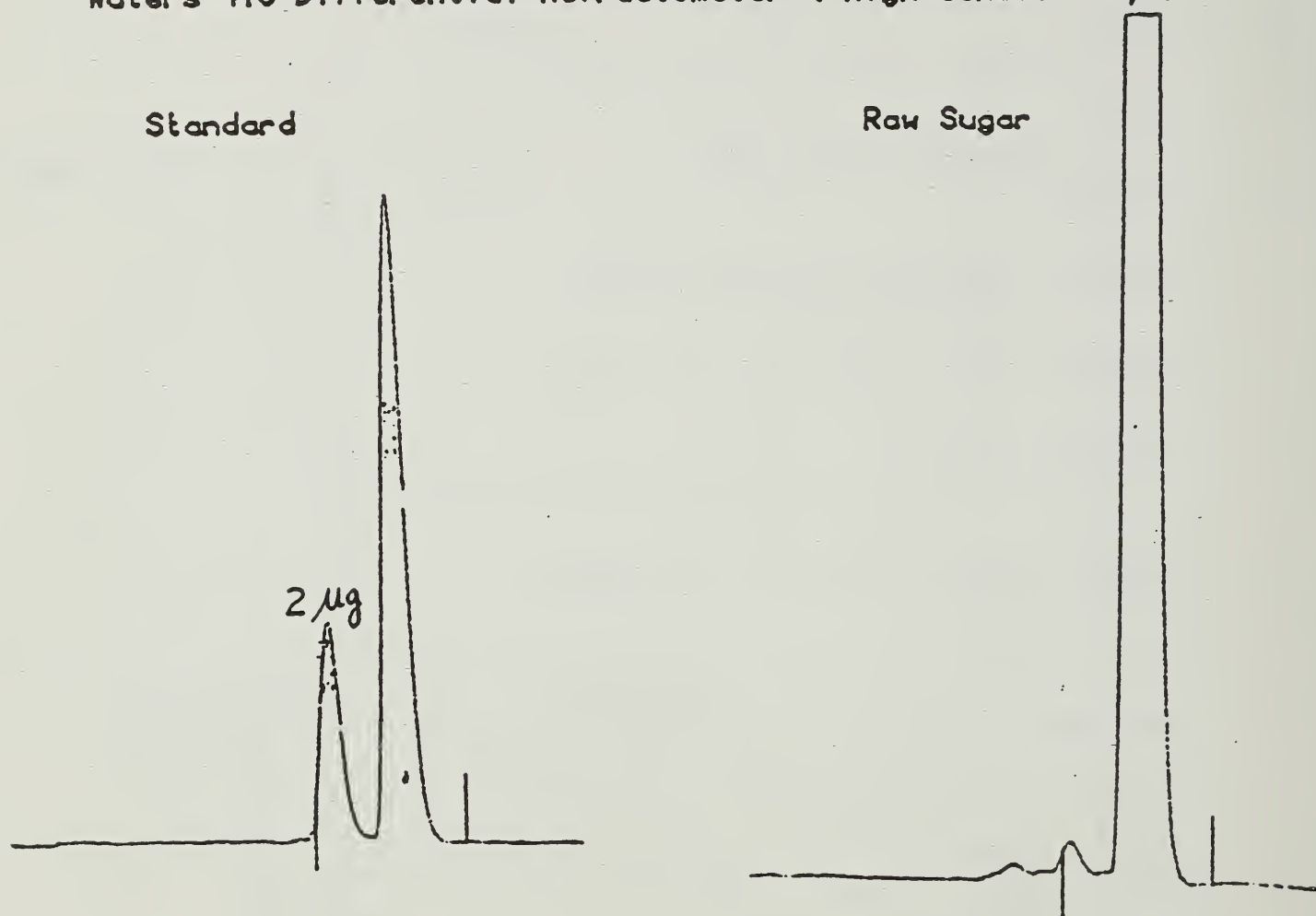
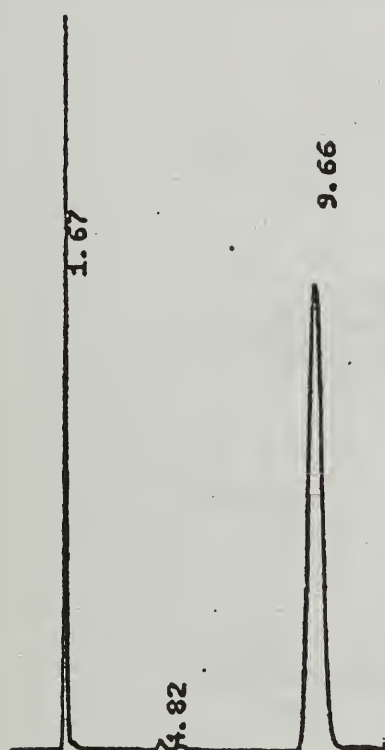


Figure 8.—Determination of invert in raw sugar by C18 Nova-Pak Column with a high sensitivity Waters 410 differential refractometer.

Anion Exchange Column +
Pulsed Amperometric Detector (Dionex)

0.025g/100ml

Raw Sugar



0.500g/100ml

Raw Sugar

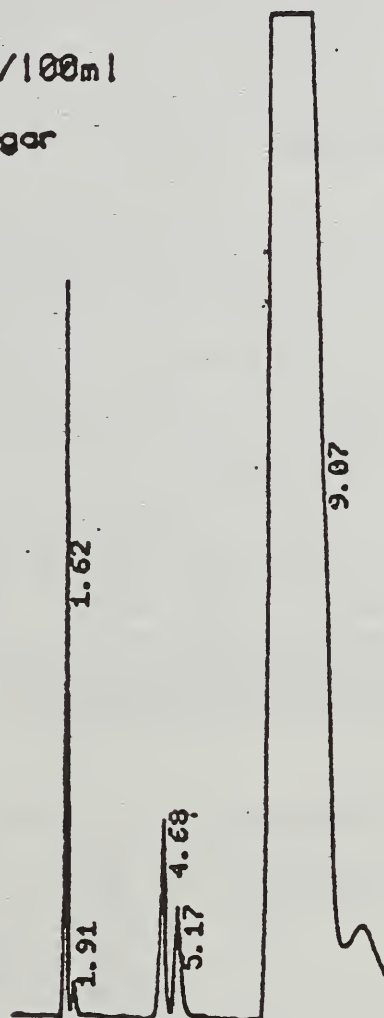


Figure 9.—Determination of invert sugars and sucrose by anion-exchange chromatography with electrochemical detection (provided by Bill Fitch, Dionex Corp.)

the analysis of carbohydrates. The combination of this chromatographic method with triple potential detection provides a highly sensitive method for complex and difficult samples. The invert in raw sugar can be separated and detected using this system (Figure 9b), an ion-chromatographic analysis.

SUMMARY

1. Although slightly less sensitive than low wavelength detectors, RI detection gives a more accurate analysis because it is less prone to interferences.
2. The Resolve C18 Radial-Pak cartridge is suitable for a rapid and routine analysis of cane juices and other sugar samples, and appropriate for use in the sugar factory laboratory.
3. Direct detection of invert in raw sugar can be accomplished using 4 μ Nova-Pak C18 columns in series with water as the eluent. The results obtained compared favorably to those of the G.C. methods. This method can be applied to the quantitative analysis of reducing sugars in other high purity sugar products.

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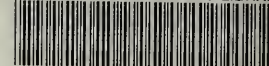
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